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VOL. 20 NOVEMBER, 1934 No. 2

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The Journal of Laboratory and Clinical Medicine

VOL. 20 NOVEMBER, 1934 NO. 2

CLINICAL AND EXPERIMENTAL

AN ABRIDGED KEY TO THE GENERA OF PATHOGENIC FUNGI*

FREDERICK W. SHAW, M.D., RICHMOND, VA.

IN PRESENTING this key for the use of clinical pathologists, the aim has been utility rather than an attempt at a natural system. No attempt has been made to place the ringworm fungi (Dermatophyta) in any systematic position, because this appears to be impossible at this time. Likewise, a few of the other pathogenic fungi have been classed as *Genera Dubia* for the same reason.

Under the *Genera Certa* have been placed those fungi whose characters are such that they may be classified, that is as far as this term may be used in relation to the *Fungi Imperfecti*.

This key contains the genera of most of the pathogens and the majority of the nonpathogens encountered by the medical bacteriologist.

A. DERMATOPHYTA. Parasitic and saprophytic forms in the skin and hair of man and other animals.

1. Parasitic

a. Conidia of one kind in culture, fuseaux (Fig. 1-a)

1. Epidermophyton

b. Conidia of two kinds

(1) Arthrospores present

(a) Aleuriospores present (Fig. 2-a); fuseaux absent

*From the Department of Bacteriology and Parasitology Medical College of Virginia. Received for publication, January 20, 1933.

2. Trichophyton

(b) Aleuriospores and hyphae with dichotomous subglobose or clavate apical branches

3. Achorion

(2) Arthrospores absent or imperfect

(a) Arthrospores absent; aleuriospores and fuseaux present

4. Microsporon

(b) Arthrospores imperfect; fuseaux absent

5. Endodermophyton

2. Saprophytic

a. Clusters of spores and mycelium in the skin

6. Malassezia

b. Nodules on the hair

7. Trichosporum

B. GENERA DUBIA

1. Cause the white grain mycetomas

8. Indiella

2. Cause the black grain mycetomas

9. Madurella

3. Cause blastomycosis and coccidioidal granuloma

10. Blastomycoides

C. GENERA CERTA (sensu lato)

1. Mycelium nonseptate

I. Phycomycetes

2. Mycelium septate 3

3. Reproduce by thallospores 4

a. Reproduce by hemispores

11. Hemispora

b. Reproduce by conidiospores 5

4. a. Reproduce by arthrospores 6

b. Reproduce by blastospores 7

5. a. Conidiophores present

1. Sterigmata (phialides) absent

II. Conidiophorales

2. Sterigmata present

VI. Phialidineae

b. Conidiophores absent

1. Conidia are aleuriospores

VII. Aleuriosporineae

2. Conidia isolated and inserted directly on the hyphae

VIII. Sporotrichineae

6. Cultures form a scum on liquid medium and yeastlike growth on solid medium

IX. Mycodermeae

7. a. Dark colored 8
   b. Bright or colorless 9
8. Conidia globose, ovate, oblong or short cylindric
   a. Conidia one-celled, continuous
      1. Conidiophores unbranched or sparsely so

12. Dematium

      2. Conidiophores branched, treelike

13. Haplographium

   b. Conidia two-celled

14. Cladosporium

9. a. Mycelium present
      1. Ascospores (Fig. 41) absent

15. Monilia

      2. Ascospores present

16. Endomyces

   b. Mycelium not present

X. Cryptococcaceae

I. Phycomycetes

A. Stolons present
   a. Sporagniophores branched; columella ovoid, narrowed at the base

17. Rhizomucor

   b. Sporangiophores not branched
      1. Sporangiophores arise at the nodes of the stolons

18. Rhizopus

      2. Sporangiophores arise at the internodes

19. Absidia

B. Stolons not present; rhizoids not present; hyphae branched

20. Mucor

II. Conidiophorales

A. Hyphae compact forming a globose, discoid or wart-shaped body.

III. Tuberculariaceae

B. Not as above
   1. Hyphae in loose cottony masses; conidia or hyphae dark, sometimes both

IV. Dematiaceae

2. Hyphae and conidia hyaline or bright colored
V. Moniliaceae

III. Tuberculariaceae

Hyphae and conidia hyaline or bright colored; conidiophores mostly dentroid or verticillate; conidia more than two-celled, fusoid, curved, rarely in chains; sporodochia with bristles.

21. Fusarium

IV. Dematiaceae

A. Conidia in chains

22. Alternaria

B. Conidia not in chains
a. Conidia dark, exogenous
1. Conidiophores simple

23. Acrotheca

2. Conidiophores whorled much below apex

24. Phialophora

b. Conidia mostly single and borne at tip, rarely clustered

25. Acremoniella

V. Moniliaceae

A. Conidia one-celled
a. Conidia in heads
1. Conidiophores unbranched

26. Cephalosporium

2. Conidiophores branched

27. Trichoderma

b. Conidia borne irregularly on hyphae

28. Acremonium

B. Conidia two-celled or more
a. Conidia two-celled
1. Conidiophores simple or nearly so; conidia capitate

29. Cephalothecium

2. Conidiophores irregularly branched

30. Diplosporium

b. Conidia more than two-celled

31. Allantospora

VI. Phialidineae

A. Conidiophores inflated at apex
a. Sterigmata unbranched
1. Sterigmata relatively long

32. Citromyces

2. Sterigmata relatively short

33. Aspergillus

b. Sterigmata branched

34. Sterigmatocystis

B. Conidiophores not inflated at apex; verticillately branched and unequal

35. Penicillium

VII. Aleuriosporineae

A. Hyphae pale, elongate

a. Sporogenous apparatus but little differentiated from the mycelium

36. Acladium

b. Sporogenous apparatus well differentiated

1. Aleuriospores colored

37. Aleurisma

2. Aleuriospores pale

38. Corethropsis

B. Hyphae dark; aleuriospores become dark

39. Glenospora

VIII. Sporotrichineae

For practical purposes there is but one genus in this group.

40. Sporotrichum

IX. Mycodermeae

A. Mycelium hyaline or bright colored

41. Mycoderma

B. Mycelium dark

42. Torula

X. Cryptococcaceae

A. Ascospores not formed

43. Cryptococcus

B. Ascospores formed

a. Spore membrane warty

44. Debaryomyces

b. Spore membrane smooth

1. Ascospores globose or ovoid

45. Saccharomyces

2. Ascospores lemon-shaped or hat-shaped

46. Willia

1. **Epidermophyton** Lang, 1879.

Hyphae and spores present in the lesions; pluriseptate spindles (fuseaux) present in the cultures; reproduction asexual, chiefly by the fuseaux. Fig. 1 organism and colony.

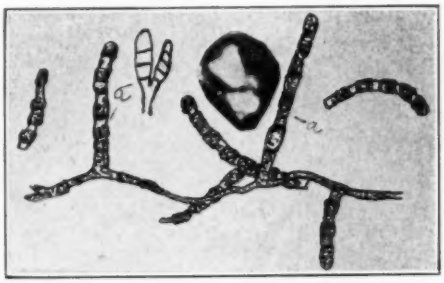

Fig. 1.—Epidermophyton.

2. **Trichophyton** Malmsten, 1848.

Hyphae and spores present in the lesions; mycelial and conidial spores in cultures; neither perithica nor asci present. The conidia are aleuriospores (Fig. 2).

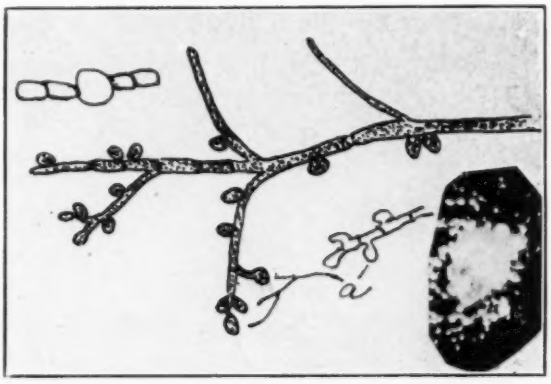

Fig. 2.—Trichophyton.

3. **Achorion** Remak, 1845.

Hyphae and spores in the lesions; spores situated apically and laterally on the hyphae, in cultures; arthrospores, aleuriospores and fuseaux in cultures in the form of clavate apical branches; without perithica or asci (Fig. 3).

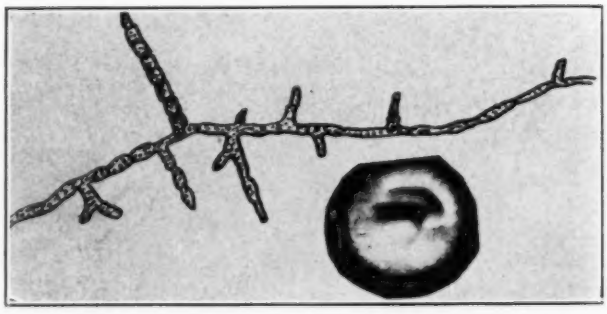

Fig. 3.—Achorion.

4. **Microsporon** Gruby, 1843.

Hyphae and spores present in the lesions; spores are small, and about from 2 to 3 u. in diameter. Aleuriospores and septate or nonseptate fusiform bodies present in the cultures. Organism in a hair and colony in Fig. 4.

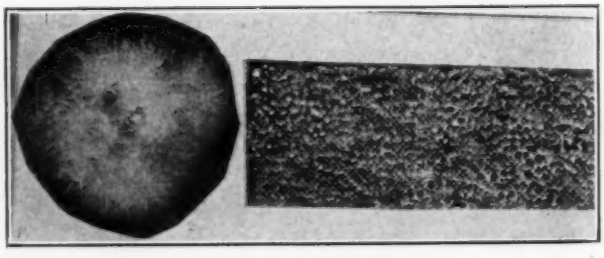

Fig. 4.—Microsporon.

5. **Endodermophyton** Castellani, 1909.

Aleuriospores rare and rudimentary; neither fuseaux nor accessary organs (spirally twisted mycelium) present; hyphae segmented; a few arthrospores may be formed.

6. **Malassezia** H. Baillon, 1889.

Members of this genus pathogenic for man have not been generally cultivated. In the tissues the organism appears as branched hyphae (segmented) and round or oval conidia, which may appear singly or in masses, and they may be smooth or have radial, longitudinal, or spiral striations (Fig. 5).

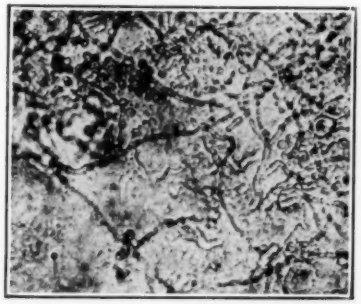

Fig. 5.—Malassezia.

7. **Trichosporum** Behrend, 1890.

Hyphae branched, hyaline then dark. Arthrospores produced. The cause of Piedra. Fig. 6 shows the lesion on the hair, the organism and colonies on dextrose agar.

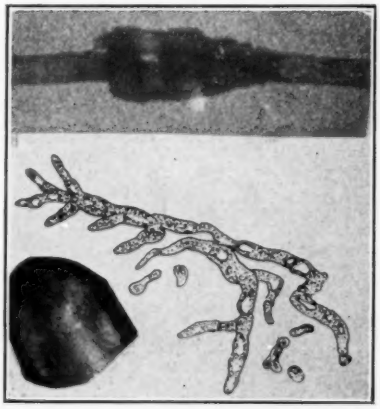

Fig. 6.—Trichosporum.

8. **Indiella** Brumpt, 1906.

Hyphae septate, branched, without black pigment. Sclerotia white or yellowish. Parasitic in the tissues of various animals. Not cultivated.

9. **Madurella** Brumpt, 1905.

Hyphae septate, reproduction by fragmentation of the hyphae. The spores are produced secondarily by binary division of the articles of which they are fragments. These fungi produce black maduromycosis. Fig. 7, organism and colony on dextrose agar.

Fig. 7.—Madurella.

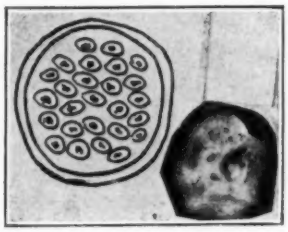

Fig. 8.—Blast. immitis.

10. **Blastomycoides** Castellani, 1927.

Budding forms appearing in the lesions as large roundish cells from 8 to 20 microns in diameter and even much larger, with the protoplasm containing a number of well-marked granules or spherules, and with the membrane showing a well-defined double contour; in dextrose agar cultures a large amount of mycelium is present. Fig. 8 shows the appearance of Blast. immitis in the tissues, and a colony on dextrose agar. Fig. 9 shows a colony, the growth magnified, and the Blast. gilchristi in the lesion.

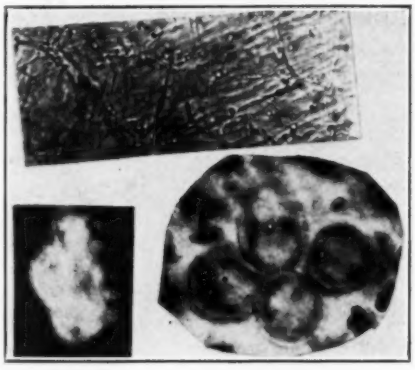

Fig. 9.—Blast. gilchristi.

11. **Hemispora** Vuillemin, 1906.

Hyphae abundant, thin, septate, branched. Fertile hyphae branched at the base. These conidiophore branches are terminated by an ampulliform structure (protoconidium) which later is transformed into a series of sporelike segments (deuteroconidia) (Fig. 10).

Fig. 10.—Hemispora.

12. **Dematium** Persoon, 1797.

Sterile hyphae creeping, little developed or forming a turf; conidiophores erect, unbranched or sparsely branched, septate, with lateral conidial chains. Conidia spherical, or ovoid, at times held together by short connecting cells; one-celled, dark colored (Fig. 11).

Fig. 11.—Dematium.

13. **Haplographium** Berk. & Br., 1859.

Sterile hyphae creeping or obsolete; conidiophores erect, with treelike cluster of branches from which chains of spores arise. Conidia one-celled, spherical or ovoid, subhyaline, olive or dark colored (Fig. 12).

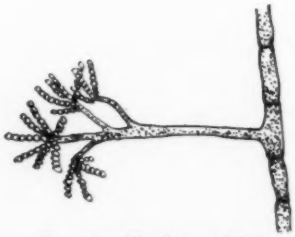

Fig. 12.—Haplographium.

14. **Cladosporium** Link, 1816.

Hyphae creeping, intricately branched; septate; olive colored. Conidiophores almost erect. Conidia in short chains or solitary, spherical and ovoid, at first one-celled, then usually two- or more-celled. Hormodendron is probably identical with cladosporium. Fig. 13 shows the hyphae and spores of cladosporium.

Fig. 13.—Cladosporium.

15. **Monilia** Gemelin, 1791.

The present tendency among medical mycologists is to include in this genus those forms which in their parasitic life form mycelial threads and budding forms, and in liquid cultures produce threads and budding forms, while on solid media the form is that of the yeasts, with very few mycelial threads. Asci are not formed. Generally produce gas from carbohydrates. Fig. 14 shows colony growth and the appearance of Monilia from a liquid culture.

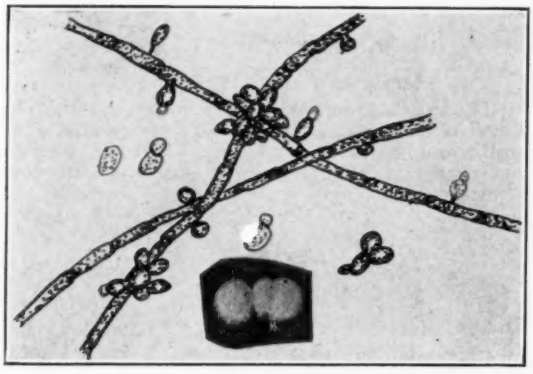

Fig. 14.—Monilia.

16. **Endomyces** Reess, 1870.

This genus has the same description as for Monilia, except that asci and ascospores are formed. Fig. 15 shows the organism from a liquid culture, a colony on maltose agar, and an ascus with four ascospores. The ascus is drawn about four times the relative size of the vegetative organism.

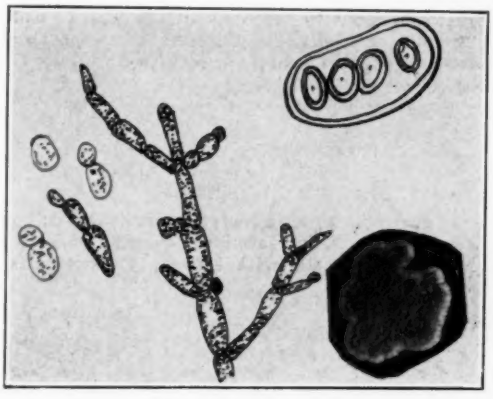

Fig. 15.—Endomyces.

17. **Rhizomucor** Lucet and Costantin, 1900.

Rhizoids and stolons present; sporophores branched; columella ovoid, narrowed at the base (Fig. 16).

Fig. 16.—Rhizomucor.

18. **Rhizopus** Ehrenberg, 1820.

Sporangiophores not branched. Stolons present. The sporangium wall is not cutinized and falls away. Sporangiophores usually arise from the nodes on which occur the rhizoids. These are generally in groups of 2, 3 or more. Columella hemispherical (mushroom-shaped). The suspensors are without appendages. Fig. 17, stolon (*S*); rhizoids (*R*).

Fig. 17.—Rhizopus.

19. **Absidia** van Tieghem, 1876.

Sporangiophores arise from the arched stolons, i. e., between the rhizoids. Columella hemispheric, conic or papilliform, more rarely terminated by a single long prolongation. Peculiar curled filaments surround the zygospores (Fig. 18).

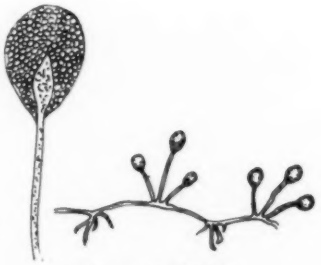

Fig. 18.—Absidia.

20. **Mucor** Micheli, 1729.

Mycelium of one kind growing in and on the substratum; sporangiophores arising singly from the mycelium but usually forming a thick turf, branched or unbranched; sporangia erect, spherical; columellae of various shape, cylindric, pyriform, clavate, colored or colorless. Spores numerous, spherical or ellipsoid with smooth surface; zygospores spherical, smooth or warty (Fig. 19).

Fig. 19.—Mucor.

21. **Fusarium** Link, 1809.

Sporodochium pulvinate or subeffused; conidiophores branched; conidia terminal, single, spindle or sickle-shaped, many cells with indistinct cross-walls (Fig. 20).

Fig. 20.—Fusarium.

22. **Alternaria** Nees, 1817.

Conidiophores fasciculate, erect, subsimple, short; conidia clavate-lageniform, septate, muriform connected by isthmi (then caudate), catenulate (Fig. 21).

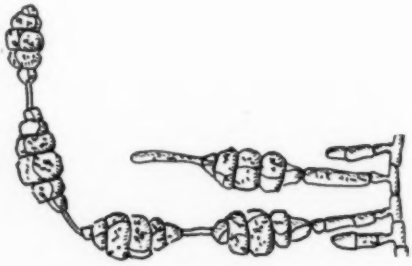

Fig. 21.—Alternaria.

23. **Acrotheca** Fuchel, 1869.

Sterile hyphae creeping, or none; conidiophores unbranched, without sterigmata, dark. Conidia fusoid or short-cylindric, dark or subhyaline (Fig. 22).

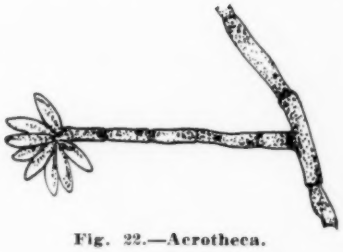

Fig. 22.—Acrotheca.

24. **Phialophora** Medlar, 1915.

Hyphae distinct from the conidia; conidiophores branched much below the apex, with short, more or less opposite branches; conidia dark, not in chains, but in heads.

25. **Acremoniella** Saccardo, 1886.

Hyphae creeping or slightly inclined, unbranched or branched, hyaline or dark colored, bearing here and there little branches, which bear conidia on the tips. Conidia spherical or ovoid, borne singly, dark, one-celled (Fig. 23).

Fig. 23.—Acremoniella.

26. **Cephalosporium** Corda, 1842.

Sterile hyphae creeping; conidiophores erect, short, not inflated at apex. Conidia sessile, spherical or ovoid, hyaline, or slightly colored, collected in heads. Fig. 24, with colony.

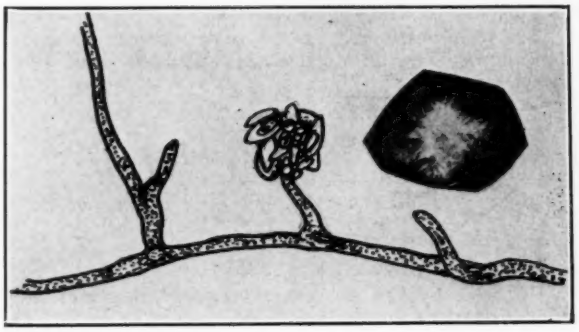

Fig. 24.—Cephalosporium.

27. **Trichoderma** Harz, 1871.

Sterile hyphae creeping, septate, forming a flat, firm turf. Conidiophores erect, arising from short, branched side-branches, branching usually opposite, not swollen at apex, conidia terminal in heads. Conidia small, mostly globose, bright colored or hyaline (Fig. 25).

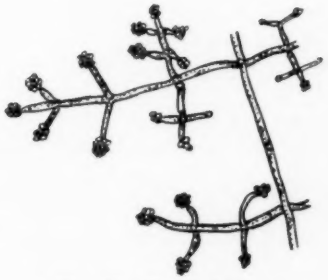

Fig. 25.—Trichoderma.

28. **Acremonium** Link, emend. Sacc. Mich., 1880.

Hyphae subsimple, procumbent; conidiophores produced here and there, simple, short; conidia solitary, hyaline or light colored, borne at the apex of conidiophore (Fig. 26).

Fig. 26.—Acremonium.

29. **Cephalothecium** Corda, 1842.

Hyphae prostrate; conidiophores erect, simple, septate; conidia apical, subcapitate, pyriform to oblong, hyaline. Identical with Trichothecium Link, 1824 (Fig. 27).

Fig. 27.—Cephalothecium.

30. **Diplosporium** Bonorden, 1851.

Sterile hyphae creeping; conidiophores irregularly branched. Conidia ovoid or oblong, two-celled, hyaline (Fig. 28).

31. **Allantospora** Wakker, 1895.

This genus is distinguished from acremonium by the conidia in the form of sausages with rounded ends. These conidia are often grouped in a head. Conidia more than two-celled, imbedded in mucus, hyaline or bright colored. Conidiophores distinct from the conidia, segmented.

Fig. 28.—Diplosporium.

32. **Citromyces** Wehmer, 1893.

Hyphae branched, septate; conidiophores unbranched; conidia on sterigmata; conidia without mucus matrix (Fig. 29).

Fig. 29.—Citromyces.

33. **Aspergillus** Micheli, 1729.

Sterile hyphae creeping, branched, septate; conidiophores erect, inflated at the apex, unbranched; conidia in chains, terminal on sterigmata. Sterigmata relatively short. Fig. 30, showing sterigma (*s*), and conidia (*c*).

Fig. 30.—Aspergillus.

34. **Sterigmatocystis** Cramer, 1869.

Sterile hyphae branched. Conidiophores erect, unbranched, distended at the apex in the form of a bladder or globule; sterigmata branched, with occasional simple forms; chains of conidia formed at the apex of the sterigmata (Fig. 31).

Fig. 31.—Sterigmatocystis.

35. **Penicillium** Link, 1809.

Sterile hyphae creeping, septate; conidiophores erect, apically irregularly verticillate-penicillately branched; conidia in chains, spherical or elliptical, hyaline or variously colored (Fig. 32).

Fig. 32.—Penicillium.

36. **Acladium** Link, 1809.

Sterile hyphae creeping, pale, elongate, septate; fertile hyphae but little differentiated; reproduction by simple aleuriospores, growing from the sides of the unbranched sporophores. Fig. 33, organism and colony.

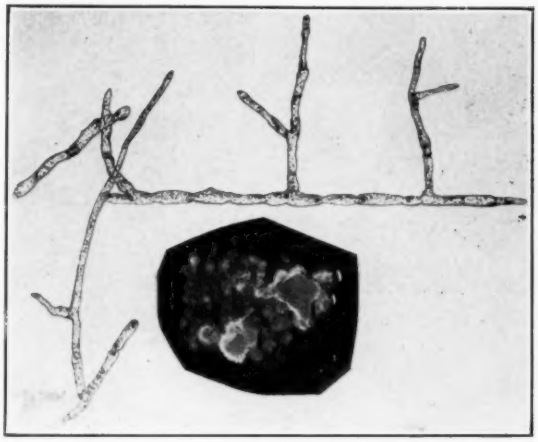

Fig. 33.—Acladium.

37. **Aleurisma** Link, 1809 (Vuillemin).

Hyphae branched, pale, elongate, septate; reproduction by simple aleuriospores; true conidiaphores absent; sporogenous apparatus well differentiated from the mycelium; spores smooth, small, colored, grow at apex and sides of the sporophore (Fig. 34).

Fig. 34.—Aleurisma.

38. **Corethropsis** Corda, 1839 (Brumpt).

Hyphae septate, elongate, branched; reproduction by aleuriospores; true conidiophores absent; sporogenous apparatus well differentiated from the mycelium; spores simple, pale, small, smooth, at the tip and on the sides of the sporophore (Fig. 35).

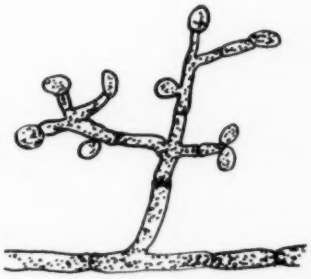

Fig. 35.—Corethropsis.

39. **Glenospora** Berkley and Curtis, 1876 (Brumpt).

Hyphae pale, dark, septate, branched, elongate; reproduction by aleuriospores; true conidiophores absent; spores simple, becoming dark on light or dark hyphae, situated at tip and sides of sporophore, small, generally 4 by 6 microns, rarely 5 by 11 (Fig. 36).

Fig. 36.—Glenospora.

40. **Sporotrichum** Link, 1809.

Hyphae hyaline, much branched; septate or continuous; conidiophores simple, short; conidia solitary or in groups on separate sterigmata, ovoid or subglobose. Organism and colony shown in Fig. 37.

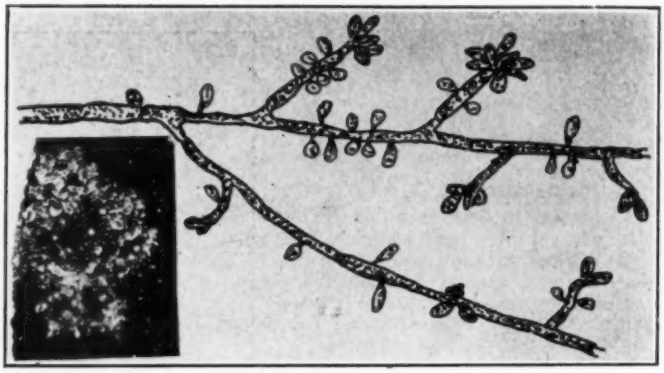

Fig. 37.—Sporotrichum.

41. **Mycoderma** Persoon, 1801.

Hyphae septate, tough, diameter greater than 1 micron. Thallus breaks up into spores (arthrospores). Form a scum on liquid mediums and yeast-like cultures on solid mediums (Fig. 38).

Fig. 38.—Mycoderma.

42. **Torula** Persoon, 1801.

Sterile hyphae decumbent; conidiophores short, scarcely different from the conidia; conidia in chains, breaking away singly or in groups; dark to black, smooth or rough, oblong to fusoid (Fig. 39).

Fig. 39.—Torula.

43. **Cryptococcus** Kützing, 1833.

Budding forms without filaments; do not produce ascospores; well-developed double contour.

44. **Debaryomyces** Klöcher, 1909.

Yeast-like organisms with the formation of single ascospores which have a warted surface (Fig. 40).

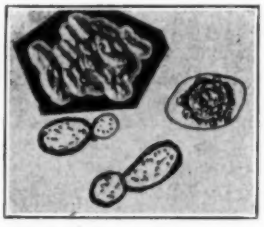

Fig. 40.—Debaryomyces.

45. **Saccharomyces** Meyen, 1838.

Budding forms with smooth ascospores. Most species form from four to eight spores. Rudimentary mycelium occasionally present. Produce alcholic fermentation. Fig. 41 shows the budding organisms and an ascus with four spores.

Fig. 41.—Saccharomyces.

46. **Willia** Hansen, 1904.

Budding forms with hat-shaped or lemon-shaped ascospores; generally do not produce alcholic fermentation; various esters produced which give characteristic odors. Form dry, wrinkled pellicles on the surface of liquid mediums. Fig. 42 shows a colony and budding cells with an ascus containing four spores.

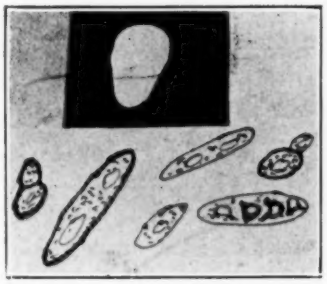

Fig. 42.—Willia.

A PHARMACOLOGIC AND THERAPEUTIC STUDY OF BROMSALIZOL, OR MONO-BROM SALIGENIN*

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INTRODUCTION

IN 1918, as a result of an extensive and intensive study of opium alkaloids in regard to the relation of their chemical structure to their pharmacologic action, one of the writers announced the discovery of interesting and valuable therapeutic properties exhibited by two chemical compounds. One of these, the ester, benzyl benzoate, exhibited a remarkably relaxant effect on the tonus of smooth muscle organs and an inhibitory action on their peristaltic movements;[1] while the other, benzyl alcohol, was found to be an efficient local anesthetic.[2] In consideration of their low toxicity, clinical observations were made with these two drugs on a selected number of patients; and the favorable therapeutic results obtained soon led to their widespread use in medicine and surgery.[3, 4, 5, 6] When these discoveries had been made public, numerous investigators immediately began a search for analogous or other closely related compounds with the object in view of improving on the therapeutic value of the original substances. Thus, for instance, Hirschfelder[7] pointed out that hydroxy-benzyl alcohol, commonly known as saligenin, which is found in Nature combined with dextrose in the glucoside, salicin, was also a local anesthetic but more stable and more soluble in aqueous solutions than phenmethylol. Again, Barbour[8] and coworkers[9, 10] noted that not only benzyl alcohol, or phenmethylol, possessed a local anesthetic action but that phenethylol and certain other closely related, nonnitrogenous aromatic compounds exerted a similar effect. With regard to the search for a more satisfactory and efficient antispasmodic drug, however, none of the compounds synthesized and tested up to this time has been found to equal in its therapeutic value the oily and rather disagreeably tasting

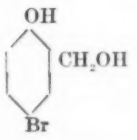
Received for publication, February 23, 1934.

*Read before the Southern Section, Society for Experimental Biology and Medicine, meeting conjointly with the Southern Medical Association, in Richmond, November 15, 1933.

ester, benzyl benzoate, which has retained its place for fifteen years in the armamentarium of the practicing physician, particularly for the relief of spastic dysmenorrhea and spastic conditions of the gastrointestinal tract. In the past four years, Dr. Fitzgerald Dunning and his associates in chemical research have been engaged in the preparation of a large number of compounds related to the benzyl esters, benzyl alcohol and to saligenin;[11] and these compounds have been subjected to a careful pharmacologic and toxicologic examination. As a result of such cooperation in research between chemist and pharmacologist, a series of compounds has been discovered which exhibit marked sedative or antispasmodic effects on smooth muscle preparations, more powerful than those produced by benzyl benzoate, and which at the same time possess certain other interesting physical and physiologic properties giving promise of possible value in practical therapeutics. These compounds have been briefly described in two short preliminary papers.[12, 13] The most interesting of these is mono-brom hydroxy benzyl alcohol, to which we have given the name of Bromsalizol; and this compound was made the object of a special intensive and extensive pharmacologic examination. Inasmuch as interesting therapeutic observations have already been gathered in this connection, it is deemed desirable to describe the properties of mono-brom saligenin in this place.

CHEMISTRY

Bromsalizol is mono-brom hydroxy benzyl alcohol, or mono-brom salicyl alcohol; that is, a halogen derivative of saligenin with the bromine atom in the para-position to the hydroxyl group. Its structural formula is represented as follows:



Analogous halogenated compounds with bromine, chlorine and iodine atoms in other positions have also been prepared and studied by us. Bromsalizol is a beautifully crystalline white powder with a melting point of from 107.5° to 109° C. It possesses a faintly aromatic odor. When applied to the mucous membranes of the mouth or tongue, bromsalizol has first a bitter taste and then produces a sensation of numbness, which is, in fact, a marked local anesthesia effected by even small amounts of the chemical; and in this respect it resembles cocaine and other well-known local anesthetics. Bromsalizol is soluble in water to the extent of 0.5 per cent and a little more readily soluble in hot water. It is readily soluble in alcohol and ether, but only slightly soluble in other common organic solvents. It can also be dissolved in oil. The drug is quite stable in both solid form and solution, and aqueous solutions do not reveal the growth of fungi because of a decided antiseptic action exerted by the compound. Although bromsalizol forms a sodium salt, when examined in this form it loses its characteristic physiologic properties. The free hydroxyl group is apparently necessary for the production of its characteristic physiologic effects.

TOXICOLOGY

A very extensive and careful study of the physiologic, pharmacologic, and toxicologic properties of bromsalizol has been carried on in our pharmacologic laboratory. Full data on the subject will be published in a more technical journal. The toxicity of this compound was tested on a large variety of living organisms—bacteria, plants, and animals. The toxicity for higher, warm-blooded animals was found to be remarkably low. The figures obtained for the dog, cat, rabbit, guinea pig, rat, mouse, and pigeon are shown in Table I. It will be noted that the lethal intravenous dose for rabbits, cats, and dogs was 250 mg. per kilogram of weight of the animals. Even large doses could be given by mouth. The largest number of toxicologic experiments were made on rabbits. It was found that the minimal lethal dose for rabbits was 0.5 gm. by mouth. We have repeatedly injected 25 c.c. per kilogram of weight of a 0.5 per cent solution of bromsalizol into the ear vein of a rabbit. Such injections had a sedative effect on the central nervous system or, at most, produced a mild stupor, from which the animals invariably recovered. We have also had occasion to administer as much as 5 gm. of the drug in food to a bear cub weighing 15.4 kilograms with no apparent effect. When fatal doses are administered intravenously to the rabbit, cat or dog, death is due primarily to paralysis of the respiratory center. After intravenous injection of massive doses of the drug, a depressant effect on the circulation is noted. After lethal doses have been administered by stomach tube, postmortem examination reveals a congested condition of the gastric and intestinal mucous membranes and a passive congestion of the kidneys.

TABLE I

TOXICITY OF BROMSALIZOL

ANIMAL	METHOD OF ADMINISTRATION	DOSE PER KILO.	RESULT
Dog	By stomach tube	1.0 gm.	Fatal
Dog	Intravenous injection	0.25 gm.	Fatal
Cat	Intravenous injection	0.25 gm.	Fatal
Rabbit	By mouth	0.5 gm.	Fatal
Rabbit	Intravenous injection	0.25 gm.	Fatal
Rabbit	Intravenous injection	0.1 gm.	Mild stupor; recovery
Pigeon	Injected under wing	0.75 gm.	Fatal
Guinea pig	Intraperitoneal injection	1.0 gm.	Fatal
Guinea pig	Intraperitoneal injection	0.2 gm.	Mild depression; recovery
White rat	Intraperitoneal injection	0.4 gm.	Fatal
White rat	Intraperitoneal injection	0.15 gm.	Coma; recovery
White mouse	Intraperitoneal injection	0.5 gm.	Fatal
White mouse	Intraperitoneal injection	0.2 gm.	Coma; recovery
Cub (*Ursus Americanus*)	By mouth	0.5 gm.	No effect

SALIENT PHARMACOLOGIC PROPERTIES

Two striking pharmacologic properties are exhibited by bromsalizol when used in even very small doses. One of these is its effect on smooth muscle tissue; the other is its action on sensory nerve endings.

A. Antispasmodic Effect.—Bromsalizol exerts a characteristic sedative action on living smooth muscle fibers and organs containing them, producing a lower-

ing of the normal tonus of the muscle tissue and an inhibition of its spontaneous normal rhythmic contractions. This can be readily demonstrated on some of the organs in the whole animal in situ and can be still more strikingly shown on all smooth muscle organs when studied in vitro by means of the surviving muscle strip preparations suspended in warm, oxygenated Locke's solution. The sedative and antispasmodic effect on the intestines can be readily observed in cats, rabbits, rats, and the dog after intravenous or intraperitoneal injections of aqueous solutions, when the abdomen is opened and the viscera are inspected with the naked eye. The relaxation of the intestines may also be observed by direct application of the solution to the serosa, or the outer coat of the intestinal wall. This is followed by a vasodilatation and relaxation of the intestinal loop. We have also demonstrated the inhibition of intestinal movements by special experiments on rats in which the passage of intestinal contents after introduction of the drug by stomach tube is measured by a special method described by one of the authors elsewhere.[14]

The best demonstration, however, of the antispasmodic and sedative or depressant action of small doses of bromsalizol on smooth muscle viscera is afforded by the study of surviving muscle preparations taken from various organs and kept in oxygenated physiologic solutions. In this way, we have performed numerous experiments on smooth muscle preparations from the intestines, uterus, gallbladder, urinary bladder, fallopian tubes, seminal vesicles, ureters, arteries and other organs from the rat, guinea pig, rabbit, cat, dog, bear, and woodchuck.

Fig. 1 illustrates the sedative effect of mono-brom saligenin on an intestinal preparation from the cat; Fig. 2 shows the lowering of the tonus and inhibition of the contractions in the uterus of the guinea pig; and Fig. 3 illustrates the relaxation and inhibition of contractions on a muscle preparation from the fundus of the urinary bladder. Such antispasmodic effects can be produced by even weaker concentrations of bromsalizol than 1:10,000. It will be seen from the illustrations that while the drug inhibits the rhythmic contractions of the organs and relaxes their spasm, this action is not equivalent to a complete paralysis and death of the tissue because the contractions can be started up again by the application of a suitable pharmacologic stimulant such as pilocarpine in case of the intestinal preparation, ergotoxin in case of the uterus, etc. Comparative studies on the relative antispasmodic efficiency of saligenin alone and mono-brom saligenin revealed that the mother substance, saligenin, by itself, possesses but very mild sedative properties for smooth muscle while the brominated derivative is from five to ten times more powerful in this respect.

B. Anesthetic Effect.—The other striking pharmacologic property of bromsalizol is its local anesthetic effect on nerve endings and nerve fibers. This local anesthetic action can be crudely detected by *tasting* the drug, when a marked numbness is imparted to the tongue and mucous membranes of the mouth. In order to ascertain more accurately the local anesthetic efficiency of bromsalizol, the writers carried out experiments by the following well-known pharmacodynamic methods:

1. *Instillation of solutions into the conjunctival sac:* Concentrations of from 0.1 to 0.5 per cent of bromsalizol, tested on the rabbit's eye by this method, exhibited a definite local anesthesia, as shown by the absence of corneal reflex.

2. *Studies on frogskin:* Reflex frog preparations were used to study the response of the legs dipped in 0.5 per cent hydrochloric acid before and after application of the drug solution in various concentrations to the skin. Such experiments also revealed that bromsalizol was a powerful local anesthetic. Solutions of 0.5 per cent produced anesthesia lasting half an hour after the frog's leg had been dipped in the drug for two minutes.

3. *Experiments by the wheal method on guinea pigs:* After shaving the hair from the abdomen of the guinea pig, a few drops of a solution are injected intradermally. The reaction of the animal to the pain produced by application of the electrodes attached to a Harvard induction coil is studied first on the

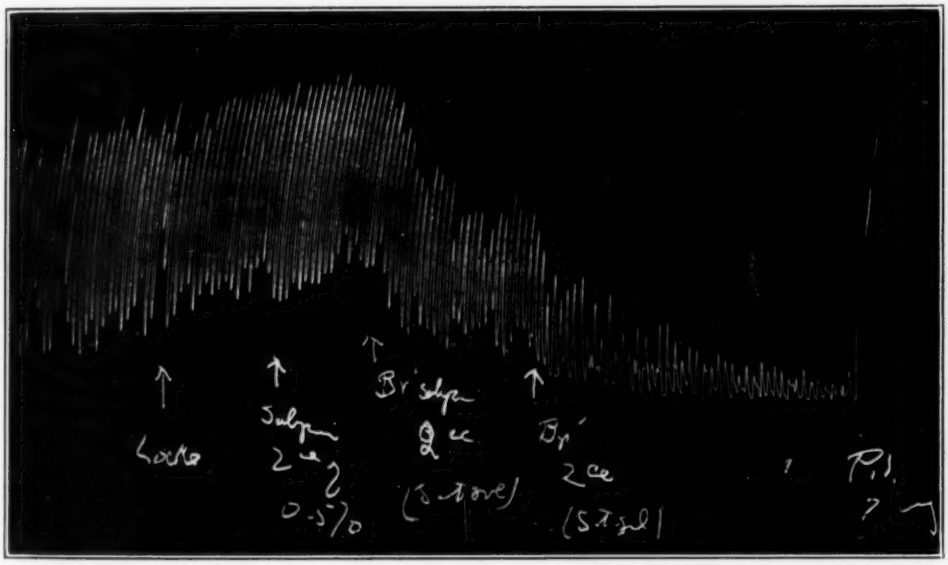


Fig. 1.—Small intestine of cat. Effect of saligenin, mono-brom saligenin, and pilocarpine hydrochloride.

normal area and then on an area in which the local anesthetic has been injected. It was found that a definite local anesthesia was produced by such intradermal injections of bromsalizol.

4. *Direct application to nerve trunks:* Solutions of the drug were applied directly to nerve fibers; and sensory and motor nerve conduction was blocked, as studied by application of electric stimuli.

5. *Experiments with direct infiltration of bromsalizol solutions in the skin, connective tissue and muscle tissue of higher animals and subsequent surgical operations on such areas:* Such injections produced a satisfactory local anesthesia so that various surgical operations were performed without inflicting pain on the animal.

On comparing the local anesthetic efficiency of mono-brom saligenin with saligen itself, both in regard to the least concentration required to produce

local anesthesia and in regard to its duration when produced by equivalent amounts of the respective drugs, it was found that the brominated compound was four or five times more efficient as a local anesthetic than saligenin alone. It was furthermore established experimentally that no undesirable synergistic

Fig. 2.

Fig. 3.

Fig. 2.—Uterus of the guinea pig. Relaxation produced by mono-brom saligenin and contraction started up again by ergotoxin phosphate.

Fig. 3.—Fundus of bladder of cat. Relaxation produced by mono-brom saligenin and contraction by subsequent dose of pilocarpine.

effects resulted from combinations of the drug with either epinephrine or ephedrine; and such mixtures effectually abolished the vasodilatation produced by bromsalizol itself by reason of its direct action on the muscle fibers.

EFFECT ON VITAL FUNCTIONS

An exhaustive pharmacologic investigation of any drug must include a study of its action on all the principal physiologic functions of different animals; and in connection with chemicals offering possibilities of therapeutic use such studies are absolutely essential before even the most cautious tests on clinical material may be undertaken. The authors have been making an intensive and extensive pharmacologic study of bromsalizol, a detailed description of which will be published in a more technical journal. In the present paper only a brief statement regarding the action of this new drug on the most important functions of the living body is given.

A. Effect on the Central Nervous System.—Small doses of the drug, comparable to those administered to patients, which will be described further on, were found to have no depressant effect of any kind on lower animals. Such studies were made particularly in psychologic experiments on albino rats trained to run in a circular maze by a method described by one of the writers in various publications.[15, 16] Doses of from 1 to 5 mg. of bromsalizol, administered by stomach or injected intraperitoneally in rats, weighing from 150 to 200 gm. on the average, produced no impairment of their behavior in the maze. Larger doses, 10 mg. or more, however, when injected in such rats, produced a mild sedation. When very large doses are injected into such animals as the guinea pig and rabbit, or given to a dog by stomach, a quieting effect is noted. Such doses, however, are many times (from 10 to 20) greater than those ordinarily used for relieving spastic conditions of internal viscera. The effect of intravenous injections is particularly noteworthy. We have injected as much as 25 c.c. of a 0.5 per cent solution of bromsalizol into the ear vein of a rabbit, and the only effect noted was a mild sedation. The animals remained quiet and were not as active as they were normally. When the effects of such injections were compared with those of saligenin, similarly administered, it was found that the latter produced very little sedation, or none at all, so that the quieting effect of bromsalizol must be chiefly ascribed to the bromine atom in that chemical compound.

B. Effect on Circulation and Respiration.—In experiments on rabbits, cats and dogs, tracings were made of the effect of the drug on the blood pressure from the carotid artery and of its effect on the respiration as studied by a method described by one of the authors elsewhere.[17] Intravenous injections of bromsalizol solutions through the saphenous or femoral veins effected the following results: From 5 to 10 mg. per kilogram of weight of the drug in single doses exhibited no effect on either the blood pressure or the respiratory curve. Small doses of from 5 to 10 mg., injected every minute, gradually produced a moderate lowering of the blood pressure without affecting in any way the strength of the heartbeat or the frequency and amplitude of the respiratory movements. Single doses of from 10 to 25 mg. per kilogram of weight of the animal, injected intravenously, produced a sharp fall in blood pressure, which returned to normal within a few minutes. After such injections the respiration was occasionally slightly slowed. Injections of 25 mg. of the drug at one time produced a more marked fall in blood pressure and a slight slowing of the

respiratory rate without interfering with either the force of the heartbeat or the *amplitude* of the respiratory movements. When such large doses as those ranging from 25 to 50 mg. were repeatedly injected, the blood pressure remained at a low level without falling further until nearly the lethal dose had been injected. When the maximum dose of the drug, which led to death of the animals, was injected intravenously, the respiration was paralyzed first and the

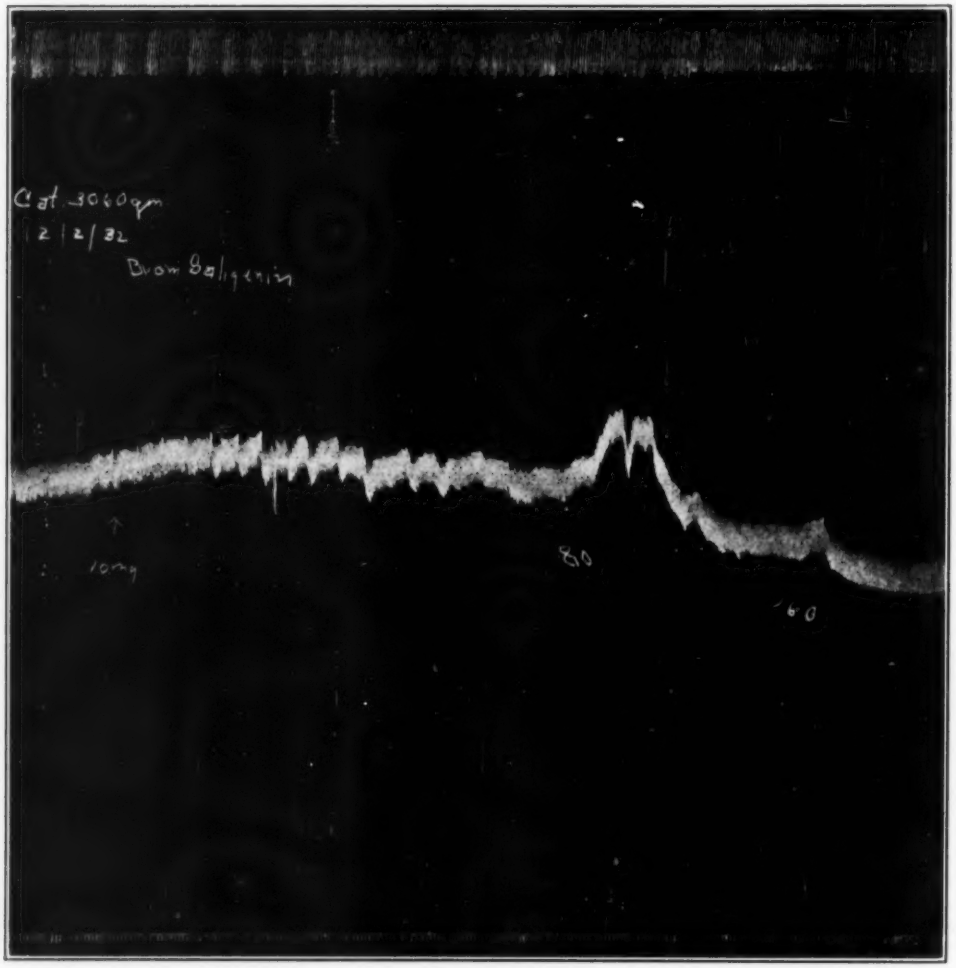


Fig. 4.—Blood pressure experiment on cat under ether anesthesia. The upper is the respiratory curve; the lower, the blood pressure curve.

blood pressure fell gradually after that to a dead level. When such animals were almost dead, they could be resuscitated by the injection of epinephrine. The fall in blood pressure noted after intravenous injections of considerable quantities of bromsalizol is, for the most part, undoubtedly due to the direct peripheral action of the drug on the smooth muscle elements in the arterial walls. This was established by means of perfusion experiments on the frog and by Krafkoff's method of perfusing the rabbit's ear. We have also found that the

drug produces a relaxation of arterial rings or strips suspended in oxygenated Locke's solution, as studied by a technic described by one of the authors elsewhere.[18] Bromsalizol, injected in doses of from 5 to 15 mg., and even more, produces no depressant effect on the vasomotor center and exhibits, if anything, a stimulating action in this respect, because the vasodilatation and fall in blood pressure are much more marked in experiments performed on decerebrated cats. The fall in blood pressure can also be demonstrated more strikingly in vagotomized animals. The effect of the drug on the heart itself was studied on excised hearts of frogs and turtles. Application of 1:10,000 solutions produced little or no effect on the heart muscle. Solutions, 1:1,000, however, when applied directly to the myocardium, either externally or by perfusion through the aorta, gradually depressed the muscle; and concentrations of 1:500 arrested the heart in diastole. Such concentrations, of course, were much greater than those obtained in the blood by administration of even very large quantities of bromsalizol through the stomach.

Briefly summarized, the effect of bromsalizol on the blood pressure and respiration in doses of from 5 to 10 mg. is nil. Slightly larger doses produce a mild lowering of the blood pressure due to peripheral vasodilatation. A depressant action on the circulation and respiration is produced only by excessive and sublethal doses of the drug, in which case paralysis of the respiratory center is followed by depression of the circulation. Fig. 4 shows the effect of monobrom saligenin on the blood pressure and respiratory curves of a cat. It will be seen that 10 mg. of the drug produced no effect on either blood pressure or respiration. Eighty milligrams produced a slightly greater lowering of the blood pressure, and 160 mg. a slightly greater fall in the blood pressure and a slight slowing of the respiration.

C. Effect on Kidney Function.—A series of experiments was performed to determine the effect of bromsalizol on the kidney function of rabbits. The drug was administered by stomach tube to some rabbits, intravenously to others, and fed in small divided doses over long periods of time to still other animals. Kidney function was studied by the Rowntree-Geraghty phenolsulphonphthalein test. It was found that unless an enormous and very toxic dose was given to a rabbit no alteration in kidney function was produced. The following records illustrate some of the findings obtained in such experiments:

EFFECT OF BROMSALIZOL ON KIDNEY FUNCTION

Rabbit No. 3; weight, 1,740 gm.:	
October 13—Phenolsulphonphthalein test; 2-hour output	90%
October 14—Given 250 mg. of bromsalizol by stomach tube	
October 19—Phenolsulphonphthalein test; 2-hour output	85%
October 23—Phenolsulphonphthalein test; 2-hour output	95%
Rabbit No. 4; weight, 1,780 gm.:	
October 13—Phenolsulphonphthalein test; 2-hour output	85%
October 14—Given 500 mg. of bromsalizol by stomach tube	
October 19—Phenolsulphonphthalein test; 2-hour output	90%
October 23—Phenolsulphonphthalein test; 2-hour output	90%

D. Effect on Liver Function.—Studies were made on the liver function in the rabbit by the Rosenthal bromsulphalein method.[19] Rabbits were daily fed from 5 to 10 mg. of bromsalizol for a week or two, and the liver function was determined at the beginning and at the end of that period. The following protocol shows the findings obtained. It will be noted that no change in liver function was produced by ingestion of bromsalizol.

EFFECT OF BROMSALIZOL ON LIVER FUNCTION

Experiment on Rabbit No. 8; weight, 2.2 kilo.:

September 11	Began feeding from 1 to 2 c.c. of 0.5% solution of bromsalizol, daily	
September 13	Bromsulphalein liver function test:	
	Reading 3 min. after injection	35%–40%
	Reading 15 min. after injection	0%
October 23	Weight of rabbit, 2.14 kilo.	
	Bromsulphalein liver function test:	
	Reading 3 min. after injection	35%–40%
	Reading 15 min. after injection less than	5%

E. Effect on Metabolism.—The effect of bromsalizol feeding on the blood chemistry of rabbits was investigated, and the results obtained are strikingly illustrated by the following protocols. It will be noted that no increase in either the blood sugar or the nonprotein nitrogen of the blood was produced in rabbits fed with bromsalizol.

EFFECT OF BROMSALIZOL ON BLOOD CHEMISTRY

Rabbit No. 5; weight, 2 kilo.:

October 9	Nonprotein nitrogen of blood	26.5 mg.
	Blood sugar	105.0 mg.
	Given 500 mg. of bromsalizol in divided doses by stomach tube	
October 16	Nonprotein nitrogen of blood	26.5 mg.
	Blood sugar	100.0 mg.

Rabbit No. 40; weight, 2.2 kilo.:

October 9	Nonprotein nitrogen of blood	36.3 mg.
	Blood sugar	88.4 mg.
	Given 500 mg. of bromsalizol in divided doses by stomach tube	
October 16	Nonprotein nitrogen of blood	29.2 mg.
	Blood sugar	86.9 mg.

OBSERVATIONS ON NORMAL MEN

After the observations on animals described above had been completed, we had an opportunity to make some studies regarding the effects of bromsalizol taken by mouth on a number of volunteers about the laboratory. Some of these subjects took only one 5-gr. dose of bromsalizol; others ingested such doses three times; and one individual volunteered to swallow from 10 to 15 gr. of the drug at one time. The men were carefully observed by one of the writers, who

made repeated measurements of their pulse rate, respiratory movements and blood pressure. The results obtained in five individuals are exhibited in the following protocols:

Subject: P. G. Blood pressure, 129/84. Pulse, 84, and respiration, 20 a minute.
Given 5 grains of mono-brom saligenin by mouth.
1½ hours later: Blood pressure, 120/82. Pulse, 88, and respiration, 24 a minute.

Subject: F. D. Blood pressure, 129/80. Pulse, 72, and respiration, 16 a minute.
Given 5 grains of mono-brom saligenin by mouth.
2 hours later: Blood pressure, 124/75. Pulse, 68, and respiration, 16 a minute.

Subject: M. F. Blood pressure, 124/78. Pulse, 62, and respiration, 18 a minute.
Given 5 grains of mono-brom saligenin by mouth.
2 hours later: Blood pressure, 112/70. Pulse, 60, and respiration, 18 a minute.

Subject: G. S. Blood pressure, 120/74. Pulse, 64, and respiration, 20 a minute.
Given 5 grains of mono-brom saligenin by mouth.
2 hours later: Blood pressure, 110/74. Pulse, 64, and respiration, 20 a minute.
Given 5 grains of mono-brom saligenin again.
2 hours later: Blood pressure, 104/70. Pulse, 64, and respiration, 24 a minute.

Subject: A. S. Blood pressure, 144/110. Pulse, 84, and respiration, 18 a minute.
Given 10 grains of mono-brom saligenin by mouth.
1½ hours later: Blood pressure, 145/105. Pulse, 96, and respiration, 20 a minute.
1½ hours later: Blood pressure 142/100. Pulse, 96, and respiration, 20 a minute.

In each case both the systolic and diastolic blood pressure was measured with a Tycos apparatus. It was found that ingestion of the drug produced practically no change in the pulse rate or in the rate of respiration. Some of the individuals revealed no change in blood pressure; more commonly, however, both the systolic and diastolic blood pressure was lowered a few millimeters. The fall was more noticeable in one individual who had a somewhat higher blood pressure than normal. As to any untoward effects of the drug, especially when taken on an empty stomach, no complaints were made. No gastric irritation was noted but some of the subjects mentioned a feeling of emptiness in the stomach in spite of the ingestion of food after the drug had been taken, an effect which must be ascribed to the local anesthetic properties of bromsalizol. The mild lowering of the blood pressure is undoubtedly due to a peripheral vasodilatation.

CLINICAL EXPERIENCES

After having studied the pharmacology of mono-brom saligenin or bromsalizol, and after having established its effective dosage, its maximal tolerated dosage, and its lethal dosage for animals of many kinds, and after having learned that it was quite harmless when given to men who volunteered to take it internally, it was deemed justifiable, and indeed desirable, in consideration of its interesting pharmacodynamic properties as an antispasmodic and as a local anesthetic, to inquire into the possibilities of the drug as a therapeutic agent in carefully controlled clinical cases. In accomplishing this end, we have been fortunate to secure the cooperation of a number of distinguished physicians, who

administered the drug to a series of patients and made careful observations concerning its action. Such clinical cases fall logically into two groups, medical and surgical.

A. Medical Applications.—Tablets containing 5 gr. of bromsalizol each were prepared and furnished to physicians for clinical use. The first few experiences revealed that the drug produced some satisfactory results, and the physicians therefore continued to administer bromsalizol to a larger number of patients. The drug was found to be particularly useful in three groups of cases: (1) gastrointestinal, (2) gynecologic, and (3) urologic.

For the first therapeutic tests with mono-brom saligenin, we are especially indebted to Professor Julius Friedenwald of Baltimore and his associates, who made a careful study of its action on a series of gastrointestinal cases. These clinical investigators found the drug efficacious in a variety of gastrointestinal disorders, all of which were characterized by symptoms referable to spastic conditions of smooth muscle organs. Patients were given 5 gr. of the drug three or more times a day. The chemical was well tolerated. There were no signs of gastric irritation after swallowing it even on an empty stomach, and no discomfort or other untoward manifestation was noted. Later, the drug was studied in other cities by a number of other well-known physicians, who also reported similar findings in gastrointestinal conditions. Up to the present time we have received reports on over five hundred clinical cases belonging to this group. The chief conditions in which relief of pain and discomfort were effected by the medicament were: pyloric spasm, spastic conditions associated with ulcers of the stomach and duodenum, gallbladder colic, spastic mucous colitis, and peristaltic unrest of the small intestine. From the pharmacologic point of view, the beneficial therapeutic effects of the drug in these cases must be ascribed in part to its antispasmodic action on smooth muscle and in part to its direct local anesthetic effect on tissues with which the drug comes in contact when introduced into the lumen of the gastrointestinal tract.

The results obtained in cases of spastic dysmenorrhea also revealed that mono-brom saligenin was equally efficient in relieving uterine colic. Here marked relief and even complete abolition of pain have been reported by a number of gynecologists who made a study of the drug. In this connection, it may be well to cite the comment of Dr. R. W. Te Linde, Associate in Gynecology, Johns Hopkins Hospital, one of the first physicians to study the drug, who administered bromsalizol to a series of cases of dysmenorrhea and in his very conservative report stated that fully one-third of the patients had been greatly relieved by the medicament, and added that those relieved had not been relieved by other drugs, such as belladonna and even codeine.

In urologic practice bromsalizol has been found useful in relieving spastic conditions of the bladder, seminal vesicles, and especially in ureteral colic. Several physicians, who reported cases of ureteral colic allayed by administration of the drug by mouth, found that the relaxation of the ureteral spasm facilitated the passage and expulsion of minute calculi. Dr. H. E. van Duzen of Dallas, Texas, has not only been employing bromsalizol by mouth but has also been using it for injection into the deep urethra in the treatment of seminal

vesiculitis and prostatitis. The results which he obtained in a series of cases were so successful therapeutically that he has prepared a paper on the subject.[20]

In addition to gastrointestinal, gynecologic and urologic conditions, in which mono-brom saligenin was found to produce favorable therapeutic results, the drug has been tried by different physicians in various other disorders characterized by spastic conditions of smooth muscle, such as coronary spasm, hypertension due to angiospasm without sclerosis of the vessel walls, a few cases of bronchial spasm, etc. The value of the drug in such cases is still sub judice.

D. Surgical Applications.—It has already been stated that mono-brom saligenin was found to be an efficient local anesthetic in laboratory experiments. In consideration of the low toxicity of the drug, it was deemed worth while to investigate its possibilities as a local anesthetic in clinical surgery. Solutions of 0.5 per cent in saline or in water, either with or without epinephrine, were prepared. Such solutions were sterilized by bringing them to a boil. It may be added, however, that bacteriologic tests with mono-brom saligenin showed that solutions of 1:200 were quite antiseptic themselves. Such solutions were furnished to a number of Baltimore surgeons, who employed them in minor surgical operations. We are particularly indebted to two surgeons for scientific cooperation in this connection. Dr. Clarence Cohn, associate of Dr. Joseph C. Bloodgood, employed solutions of bromsalizol in minor surgical operations and reported that the drug was equally as effective as novocaine. An even larger series of cases has been studied by Dr. Elliott H. Hutchins at the Mercy Hospital, Baltimore, and in his office practice. He reported that the drug gave complete satisfaction as a local anesthetic in general surgery. In dental surgery, the results obtained were not so clear-cut. Dr. Emanuel Krieger,* dental surgeon, performed a number of extractions with complete anesthesia after injecting bromsalizol along the nerve sheathes. In other cases, however, he found that the local anesthesia thus obtained was not as effective as that produced by cocaine. Another useful application of bromsalizol as a local anesthetic has been made by Dr. Victor F. Cullen of Maryland State Sanatorium. Dr. Cullen found that strong solutions (from 25 to 40 per cent) of bromsalizol in alcohol, when applied topically to tuberculous nodules and ulcers of the larynx, produced a complete anesthesia, which enabled him to cauterize the affected areas without pain.

SUMMARY

The pharmacologic, toxicologic, and therapeutic properties of a new drug, mono-brom saligenin, named Bromsalizol, have been carefully investigated. Two striking pharmacologic properties are exhibited by this chemical—a powerful antispasmodic or relaxant action on smooth muscle fibers and organs containing them, and a definite local anesthetic effect on sensory nerve endings and nerve fibers. These two salient pharmacodynamic effects are produced by doses of the drug far below the toxic concentrations, thus leaving a wide margin of safety for its therapeutic testing on higher animals and man. Doses of from 5 to 10 gr. may be taken three or four times daily with impunity. The pharmacologic effects described above have been substantiated by cautiously conducted

*Died September 12, 1933.

clinical tests on a selected number of patients, and the results obtained reveal its effectiveness as a therapeutic agent in medical cases characterized by spastic conditions of smooth muscle viscera in gastrointestinal, gynecologic, and urologic disorders, on the one hand, and as a local anesthetic in minor surgery, on the other. The data already collected render a further and more extensive clinical therapeutic investigation of the drug very desirable, and such a study is in progress.

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THE CLINICAL SIGNIFICANCE OF A VERY LOW CONCENTRATION OF UREA IN THE BLOOD*

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THE normal range of the concentration of urea in the blood has been accepted as approximately 10 to 50 mg. per cent.[1] The lower values of the normal range have been found in individuals on a low protein intake, a high urine output, or on both. In the last few years one of us (Osterberg) has observed, periodically, in our central clinical laboratory, a blood urea content below 10 mg. per cent. On further investigation of the clinical findings in such a case, the diagnosis was frequently found to be that of chronic wasting disease. In one such case active, chronic diffuse pulmonary tuberculosis was known to have been present, but there was also a complicating renal disturbance. Naturally we were surprised to find that the urea content of the blood in this case was only 6 mg. per cent. These isolated examples of a low urea content of the blood led us to go over the laboratory records for the last four years, and, as a result of the survey, we found 25 cases with widely differing ailments in which the concentration of urea in the blood was less than 10 mg. per cent (Table I). We have been unable to find in the literature a report of a similar series of such low values either in normal individuals or in patients suffering from various diseases.

Method of Determining Urea.—For the determination of urea in the blood we have used the van Slyke and Cullen[2] modification of the Marshall urease method on whole, oxalated blood. A preparation of the enzyme of soy bean (arlco-urease) was used and the aeration continued for forty-five minutes. In every instance when values below 10 mg. per cent of urea in the blood were encountered, the determination was repeated several times and always checked.

A chance technical error may be responsible for a low value for urea in the blood in any large series of analyses; however, we believe that these extremely low values for urea are not due to technical error for the following reasons: (1) when a very low value was initially obtained the determination was checked several times; (2) a low value could not be attributed to a decreased activity of the urease enzyme because, simultaneously with these analyses, many analyses employing the same solution of urease were performed on blood with normal and high values for urea, and (3) in Case 1, repeated estimations of urea in the blood over a period of two weeks revealed values of 6, 8, 12, and 10 mg. per cent.

Clinical Data.—Several patients were suffering from a serious chronic disease, such as pulmonary tuberculosis, encephalitis, diffuse skin lesions,

*From the Mayo Clinic.
Submitted for publication, February 20, 1934.

chronic suppuration, diabetes mellitus, duodenal ulcer, and Addison's disease. In some cases also there were chronic renal lesions, these including bilateral pyelonephritis, bilateral hydronephrosis, tuberculosis of the kidneys, and the diffuse nephritis associated with disseminated lupus erythematosus. The occurrence of a low value for urea in the blood in diabetes insipidus would seem to be a possible result of the enormous water exchange. Similarly, in diabetes mellitus, the low value for urea might be the result of polyuria. In duodenal ulcer with obstruction the water and inorganic metabolism may be markedly upset, but it can readily be corrected by large intravenous injections of fluid. In this condition also a low value for urea in the blood might be the result of a large intake of water. However, in other cases no definite organic lesions or marked physiologic disturbances were demonstrated, and the symptoms were described as being due to nervous exhaustion (see Table I).

TABLE I

TWENTY-FIVE DIFFERENT CASES OF DISEASE WITH CONCENTRATION OF UREA IN THE BLOOD LESS THAN 10 MG. PER CENT

CASE	AGE, YEARS, SEX	WHOLE BLOOD UREA, MG. PER CENT	DIAGNOSIS
1*	24 F	6	Chronic pulmonary tuberculosis, acute pericarditis
2	56 F	8	Chronic pulmonary tuberculosis
3	34 F	8	Tuberculosis of left ovary
4	43 F	8	Left tuberculous kidney, tuberculous cystitis
5	41 F	8	Bilateral hydronephrosis
6	48 F	8	Chronic pyelonephritis
7	68 M	9	Postoperative infection, prostatectomy
8	25 F	6	Disseminated lupus erythematosus, chronic nephritis
9*	28 F	8	Stricture common bile duct, obstructive jaundice
10	25 M	8	Extensive infected burns
11	10 M	8	Lateral sinus thrombosis, pulmonary abscesses
12	44 F	8	Duodenal ulcer, gastroenterostomy
13	63 M	9	Chronic prostatitis and suppuration of pleura
14	30 F	8	Addison's disease
15	31 M	6	Extensive psoriasis
16	39 F	6	Diabetes mellitus
17	16 M	9	Diabetes insipidus, encephalitis
18	44 F	8	Lethargic encephalitis
19	32 F	8	Pregnancy, hypotension
20	64 F	8	Chronic urethritis, obesity
21	20 F	6	Angioneurotic edema
22	44 F	8	Chronic nervous exhaustion
23	57 F	8	Chronic nervous exhaustion
24	45 F	9	Chronic nervous exhaustion
25	58 F	8	Chronic diffuse fibrositis and chronic nervous exhaustion

*Necropsy.

MacKay and MacKay[1] have shown that the normal concentration of urea in the blood tends to be distinctly lower in the female than in the male. Their lowest figures, 11 to 20 mg. per cent, always occurred in females. In the present series, nineteen, or 76 per cent, were females, and our lowest concentration, 6 mg. per cent, was found in four females and in only one male. Such findings indicated that a low concentration is more likely to occur in the sick, as well as in the normal, female.

The importance of the effect of a high or low protein, and a large or small water intake on the concentration of urea in the blood is well recognized. In Case 17, the water intake and urine output reached as much as 16,000 c.c. in twenty-four hours. Thus, one might expect a washing out of urea. To a less extent the increased water exchange in diabetes mellitus might have a similar, but less marked effect. In a wasting disease with anorexia and vomiting, there may possibly be a decrease in the formation of urea and a gradual washing out of the urea already present. This is illustrated in Case 1. Repeated estimations of urea in the blood over a period of two weeks revealed values of 6, 8, 12, and 10 mg. per cent. During this period there was a distinct increase in the intake of food. A similar explanation applies to Cases 2 and 9.

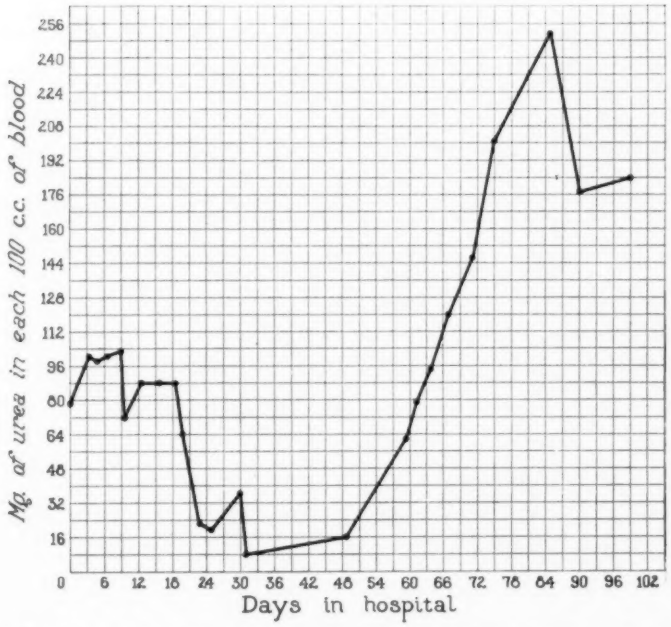

Fig. 1.—Case 8. Disseminated lupus erythematosus, chronic nephritis.

The occurrence of an abnormally low content of urea in the blood in cases with bilateral renal disease seems rather paradoxical. In Case 8, one of disseminated lupus erythematosus, both before and after the very low value for blood urea was found, there was evidence of severe renal insufficiency (Fig. 1). At the period when the value for urea was abnormally low, 6 mg. per cent, the patient was taking in and excreting a considerable volume of water, but the diet was inadequate. The most plausible explanation is that during a temporary process of healing in the kidneys, urea and water were readily excreted and the production of urea was decreased. In this connection it is of interest to note that in Case 1, with chronic pulmonary tuberculosis, albuminuria and cylindruria developed during the last month of her illness, that the value for urea in the blood was extremely low, 6 mg.

per cent, and, at necropsy, there were no demonstrable histologic abnormalities in the kidneys. Thus, it is possible to have a very low value for urea in patients with abnormal kidneys, whether the renal disturbance be due to demonstrable histologic change or to pathologic physiology.

COMMENT

An abnormally low concentration of urea in the blood can occur in various diseases. Paradoxical as it may seem, it has been observed in bilateral renal disease. The simplest explanation of such a finding is that there may exist a decreased production of urea because of a disturbed nitrogen metabolism, or an enhanced excretion of urea by the kidney due to increased fluid exchange. Both of these factors may be involved.

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CHOLESTEROL ESTERS AS A MECHANISM OF FAT METABOLISM*

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THE following paper is a summary of 67 cases and 93 determinations of cholesterol ester ratios. These cases are taken from the wards of the Bronx Hospital.

The object of this investigation is to determine the significance of the cholesterol ester ratio in diabetes, liver, and kidney diseases.

Method.—The total cholesterol was determined on blood plasma, using the Liebermann-Burchard reaction. Bloor's mixture of alcohol and ether was used in the extraction. The esters were determined by combining the free cholesterol with digitonin, evaporating off the alcohol and ether and extracting the esters with petroleum ether. The petrolic ether extract was evaporated and the residual esters were taken up with chloroform; the final determination was the same as for total cholesterol.[1]

Cholesterol Esters.—The normal cholesterol of the blood plasma varies in individuals from 150 to 200 mg. per 100 c.c. Bruger and Somach[2] found the cholesterol varied in four normal individuals from 172 to 264 mg. per 100 c.c. The variation in one individual was plus or minus 8 per cent in twenty-four hours.

Bloor[3] states that under abnormal circumstances the cholesterol level may rise or fall, but in health the cholesterol content is fairly constant. Muller[4]

*From the Laboratories of the Bronx Hospital, New York City.
Received for publication, February 28, 1934.
I wish to acknowledge my indebtedness to Dr. J. Felsen, director of Laboratories and Medical Research, for his suggestions and advice in the preparation of this paper.

says that cholesterol is synthesized in the body and eliminated through the bile and is reabsorbed through the intestines. Cholesterol synthesis is associated with the adrenals, liver, and spleen. Knudsen[5] states that the esters of cholesterol exist as palmitic, stearic, and oleic acid esters. There is a definite balance between free and bound cholesterol in health and under pathologic conditions. The cholesterol as ester is from 46 per cent to 70 per cent of the total. Strobe[6] found from 56 per cent to 80 per cent of the total is normal for esters.

Leites and Goldbitz[7] describe a test meal of 50 c.c. of olive oil. There is a normal rise of from 8 per cent to 44 per cent in the esters during the first seven hours after ingestion of this meal in normal patients. Failure to give this change indicates catarrhal icterus, cholecystitis, and cirrhosis. Table I indicates no marked change in the ester ratio. This may be a good test, but the discomfort of the patient with the aforementioned pathology precludes the use of this test. Most of these patients will refuse oil or fat.

TABLE I

PATIENT INGESTED 50 C.C. OF OLIVE OIL: CHOLESTEROL AND CHOLESTEROL ESTERS IN MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	DATE	CHOL.	ESTERS	PERCENTAGE	TIME AFTER INGESTION
49 E. S. Diabetes	11/20	275	64	23	
	11/27	157	108	68	Fasting
	11/27	136	92	67	4 hr.
	11/27	162	122	73	7 hr.
53 B. V. Diabetes	11/22	227	166	73	Fasting
	11/22	222	156	70	1 hr.
	11/22	227	150	66	2 hr.
56 D. Normal	2/ 4	166	113	67	Fasting
	2/ 4	171	123	72	4 hr.

Cholesterol Esters in Liver Disease.—Strobe[6] states that as jaundice of hepatic origin begins, esters decrease from 13 to 43 per cent from the normal. A fall of cholesterol-ester ratio is good evidence of hepatic disease. Adler and Lemmel[8] state that a lowering of the values is parallel with the severity of acute, subchronic yellow atrophy and icterus catarrhalis; and with recovery normal values return. Table II is a series of determinations made on patients with suspected liver involvement.

Case 14 gives lowered ratios of 35 per cent and 57 per cent two weeks later. Cases 59 and 60 give lowered ratios. The cholesterol-ester ratio does not parallel the icteric index in these cases. In Case 14 the ester ratio returns to normal while the icteric index indicates a more severe jaundice at the time of the second determination. All these patients were on a fat-free diet and where the icteric index indicates possible liver damage, the therapy consists of intravenous glucose. Epstein[9] states that the ester values of the blood plasma in acute parenchymatous damage of the liver are diminished or reduced to zero in proportion to the severity of the disease. The partition is normal in atrophic cirrhosis. Two cases of cirrhosis, Table IV, Case 23, and Table II, Case 16, give normal results. In conclusion, the ester ratio is related

TABLE II

CHOLESTEROL AND CHOLESTEROL ESTERS IN MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	CHOL.	ESTERS	PER-CENTAGE	DIAGNOSIS	ICTERIC INDEX
3	402	234	58	Chronic cholecystitis	Normal
5	230	140	61	Acute cholecystitis and catarrhal jaundice	36
	222	140	63		17
	190	110	58		10
10	256	134	52	Chronic cholecystitis and choleliathiasis	17
11	190	123	65	Chronic cholecystitis	9.3
	190	124	65		
13	225	139	62	Acute cholecystitis	
14	285	98	35	Jaundice	94
	266	153	57		112
16	186	119	64	Cirrhosis	10
18	189	108	58	Chronic cholecystitis	6.2
21	188	122	65	Chronic cholecystitis	
	170	154	90		
31	230	170	74		9.8
33	400	275	69	Carcinoma of bile duct	72-180
35	231	143	62	Carcinoma?	32
	250	166	66		80
38	183	145	78	Acute cholecystitis	
40	204	127	62	Cholecystitis	9.1
47	207	144	69	Cholecystitis	7.2
55	177	120	68	Jaundice	46
59	148	65	43	Pneumonia and icterus	84
60	250	85	34	Niemann-Pick's disease	Normal
22	242	140	58	Generalized tuberculosis	

to liver disease, but the absence of fat from the diet and the injection of intravenous glucose seem to interfere with the ratio.

Cholesterol Esters in Diabetes.—Bloor[3] quotes a series of abnormal cholesterol-ester ratios in diabetic patients. Table III, Case 49, is the only abnormal cholesterol-ester ratio which the author has been able to find.

Case 49 had not been treated by diet or insulin at the time of the first determination. This same patient one week later, as shown in Table I, gives a normal cholesterol ester ratio after she was advised on her diet. These results correlate well with the work of Best.[13] He showed that dogs with extirpated pancreas on a normal fat diet deposited fat in the liver. If they

TABLE III

CHOLESTEROL AND CHOLESTEROL ESTERS AS MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	CHOL.	ESTERS	PERCENTAGE	DIAGNOSIS
1	222	125	56	Diabetic gangrene
4	217	135	62	Diabetic gangrene
	216	139	64	
19	261	166	60	Diabetes
24	354	241	68	Diabetes and chronic arthritis
	286	178	63	Diabetes and coronary thrombosis
20	255	167	65	
32	244	170	69	Diabetes and cystocele
49	275	64	23	Diabetes, untreated
50	170	104	61	Diabetes
51	207	150	72	Diabetes
52	264	194	73	Diabetes
53	227	167	73	Diabetes
54	215	112	52	Diabetes and uremia

are given insulin or are subjected to a fat-free diet, they do not manifest this fatty deposit in the liver. Chaikoff and Kaplan show a lowered ester ratio in experimental diabetes.[14] It is not uncommon to find fatty deposits in diabetic patients at autopsy. The indications are that insulin, or a fat-free diet, prevent the lowering of the cholesterol-ester ratio and the deposition of fat. The cholestrol-ester ratio, as observed from fatty degeneration of the liver in parenchymatous liver disease and the lowered cholesterol-ester ratio observed in diabetes, point to a close relationship between glycogen, glucose, and cholesterol esters. The carbohydrates necessary for the combustion of fats are lacking in the tissues in diabetic patients. This leads to disturbed fat metabolism. This disturbance manifests itself by the lowered cholesterol-ester ratio.

Cholesterol Ester in Renal Disease.—Epstein and Lichtenstein[10] quote 11 cases of nephrosis with increased cholesterol-ester ratios. The cholesterol-esters are from 80 per cent to 90 per cent of the total in 6 cases. Two cases give cholesterol esters of 36 per cent and 38 per cent. One of these gives a 90 per cent ratio twelve days later.

Table IV shows three cases of true nephrosis with abnormal albumin-globulin ratios.

TABLE IV

CHOLESTEROL AND CHOLESTEROL ESTERS IN MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	CHOL.	ESTERS	PER-CENTAGE	DIAGNOSIS	BLOOD CHEMISTRY
8	375	150	40	Nephritis and nephrosis	N.P.N., 70-200
	275	137	49		Albumin-globulin ratio 3/1 and 1/1
	276	156	56		
23	145	92	64	Uremia	Urea N, 41
26	162	57	35	Acute nephritis	Urea N, 33; A/G, 1/1
29	750	461	62	Nephrosis 1 wk.	A/G/0.3/2.2
30	292	231	79	Nephrosis	A/G/1/2
43	143	83	58	Sepsis	N.P.N./50
48	383	333	84	Nephrosis	A/G/1/1
	345	250	72		
54	215	112	52	Uremia	N.P.N./250
58	130	70	54	Intest. obstruct.	Urea N, 88
44	163	120	73	Nephritis	Urea N, 14
62	114	69	60	Nephritis	N.P.N., 46.9

Case 8 was originally diagnosed as nephritis with a normal albumin-globulin ratio of 3 to 1 and cholesterol-ester ratio of 40 per cent. Two months later the patient showed nephrotic symptoms with an albumin-globulin ratio of 1 to 1, and a normal cholesterol-ester ratio. The N.P.N. rose from 70 to 200 at the time of patient's death. Case 26, that of a child, was diagnosed as acute nephritis, with an albumin-globulin ratio of 1 to 1 and a cholesterol-ester ratio of 35 per cent. Cases 30 and 48 are in agreement with Epstein's and Lichtenstein's findings. The conclusion which may be drawn is that if the nephrosis is of nephritic origin, the cholesterol-ester ratio seems to rise from subnormal to above normal. The initial rise of cholesterol depresses the ester ratio and we find fatty deposits in the kidney, due to the lowered ratio. This phenomenon along with the disturbed protein metabolism may be related to the edema of nephrosis. Calvin and Goldberg[11] state that

the hypercholesterolemia of renal disease is probably the result of a disturbance in fat metabolism accompanying the nephrotic syndrome, rather than the cause or result of edema. The cholesterol content of the blood during edema is higher than normal and may remain high after edema has disappeared. The cholesterol values vary directly with the degree of edema, the changes in cholesterol being preceded by the appearance or disappearance of edema. The cholesterol appears to be mobilized from deposits of fat since the edema is usually associated with emaciation and the blood cholesterol remains high. The fatty deposit in the nephrotic kidney may be compared to the fatty deposit in the liver of acute jaundice, both resulting from a lowered cholesterol-ester ratio.

The accompanying diagram is a simple scheme for showing the relationship between glucose and cholesterol esters in the blood and tissues.

Tissues		*Blood*		*Extraneous*
Neutral Fat	⇄	Cholesterol Esters	⇄	Fats
Glycogen	⇆	Glucose	⇆	Sugar

There seems to be an equilibrium between neutral fat and glycogen in the tissues; cholesterol esters and glucose in the blood. The neutral fat is conveyed by means of the cholesterol esters. Sobotka[12] designates the cholesterol esters as a transportation form of fats. A disturbance of tissue glycogen, as in diabetes, would shift the equilibrium of tissue glycogen to blood glucose to glycosuria, which I designate as extraneous, or outside the body. The fat equilibrium shifts from ingested extraneous fat to cholesterol esters to a deposition of neutral fat in the tissues. The cholesterol esters fall below their normal percentage, and deposit the fats in the tissues. Insulin seems to reverse this process, causing a retention of glucose and glycogen. The cholesterol esters increase, drawing fat from the tissues.

In fatty degeneration of the liver, that is, acute or subchronic parenchymatous liver disease, the deposit of fat in the liver (Kupffer cells) causes the retention of bilirubin in the blood. Intravenous glucose shifts the carbohydrate equilibrium to the left and the fat equilibrium to the right. In other words, this phenomenon seems to resemble the fat disturbance in diabetes. It also seems significant that jaundice follows a disease which calls on the reserve glycogen, such as pneumonia and influenza. The injection of glucose seems to mobilize the fat from the liver and the cholesterol-ester ratio returns to normal. The icteric symptoms disappear and the liver condition clears up. In nephrosis the emaciated state of the patient is probably due to low glycogen as well as the loss of protein. The diagram indicates a deposit of neutral fat (in the kidney) and a low cholesterol-ester ratio in the initial stages. The high cholesterol-ester ratio of the later stages represents an attempt on the part of cholesterol to remove the fat from the tissues. Epstein and Lichtenstein[10] describe the appearance of fat bodies and cholesterol esters in the urine of nephrotic patients. This points to a complete shift of the equilibrium to the right in the above diagram.

CONCLUSION

The cholesterol-ester ratio is abnormal in diabetes and parenchymatous liver disease only under certain conditions of diet and therapy. It is abnormal in nephritis and nephrosis. The ester ratio seems to be closely related to carbohydrate metabolism. A simple diagram illustrating this relationship is shown.

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PRIMARY CARCINOMA OF THE DUODENUM*

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THE duodenum is one of the rarer sites of primary neoplastic growth but cases are reported with increasing frequency. Several extensive reviews are now available, the recent ones of Eusterman, Berkman, and Swann,[1] Meyer and Rosenberg,[2] and Mateer and Hartman[3] being especially complete and presenting both the clinical and pathologic features.

Since their publications several other cases have been reported. Involving the supraampullary region are two cases of Arisz[4] and one each of Pacetto,[5] Rutishauser,[6] and Bookman.[7] The first case of Arisz is clearly one of cancer developing in chronic ulcer. Rutishauser's case presented multiple tumors originating in Brunner's glands and had peritoneal metastases. The carcinoma in Bookman's case originated in aberrant pancreatic tissue. Rutishauser also reported five periampullary carcinomas. Forni,[8] Giordano,[9] and Marcus[10] each had one case involving the infraampullary region. Marcus' case is of particular interest. Seven years after the removal of a stenosing adenocarcinoma of the duodenum multiple adenomas of the stomach were discovered at operation. He suggests that the basic condition was an adenomatosis of stomach and duodenum with malignant degeneration in the duodenal region. He could find no other instance in the literature of a case similar to his own.

In the Pathological Department of City Hospital from May 24, 1911, to Dec. 31, 1933, there were 358 cases of carcinoma in 3,956 autopsies. Two were primary carcinomas of the duodenum and are the subject of this report.

CASE REPORTS

CASE 1.—The patient, a white woman fifty years old, was admitted to the service of Dr. E. P. Shelby because of extreme weakness and night sweats. For one month she had become extremely weak, had lost considerable weight and had had severe night sweats. The most striking feature was the extreme lethargy. There was general muscular atrophy. In the left epigastrium was an ill-defined sense of fullness and resistance. Jaundice was absent. The urine contained a faint trace of albumin. Gastric contents: free HCl 20, combined HCl 36, cellulose, yeast starch and fat. Duodenal drainage was unsuccessful, only bile-tinged gastric contents being obtained. Roentgenology revealed an orthotonic stomach without defects, normal duodenal bulb and high fixation of the second duodenal segment.

Course: The course was characterized by marked weakness, intense lethargy deepening into coma, the development of progressive jaundice, bilirubinuria, severe anemia and a low grade septic fever. Vomiting was never present. The only physical findings of note were increasing size of the liver, edema of the abdominal wall, and terminal bronchopneumonia. Death occurred one month after admission and two months after the onset of the illness.

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Received for publication, February 13, 1934.

Anatomical diagnoses: primary carcinoma of the periampullary portion of the duodenum; acute suppurative cholangitis; chronic interstitial pancreatitis.

Fig. 1.—Case 1. A view of the duodenum opened anteriorly. At the papilla is a small excavated ulcer. The common bile duct is dilated.

Fig. 2.—Case 1. Photomicrograph of the section through the ulcerated surface, showing the malignant adenomatous structure with marked papillary formation.

The peritoneal cavity contained 200 c.c. of a clear bile-tinged fluid. At the papilla was a small soft friable ulcer 2 cm. in diameter. The duodenum, stomach, common duct and gallbladder were distended.

Histology.—The ulcerated papilla was covered by a necrotic membrane. The edges and adjacent duodenal mucosa had a neoplastic epithelium of high columnar cells characterized by papillary formation. The nuclei were hyperchromatic and mitoses were frequent. Lymphoid reaction was moderate. The lower ends of the pancreatic and bile ducts emptied into the ulcer base; their epithelia were lost by postmortem desquamation but the underlying stroma was normal and free of malignant cells. Metastases were absent.

CASE 2.—The patient, a white male of thirty-four, was admitted to the service of Dr. W. L. Whittemore. One year before he had high substernal pain referred soon after the onset to the eleventh dorsal spine and later to the epigastrium. For a long time it was diffuse and not related to meals. On Christmas a half hour after a hearty meal, he suffered severe epigastric pain relieved by vomiting. Since then similar attacks recurred, the pain radiating

Fig. 3.—Case 2. Gross specimen of duodenum, pyloric region and pancreas. The pylorus is normal. The arrows indicate the upper border, the spear heads the lower border of the carcinoma. The papilla of Vater projects prominently below the lower border. The common bile duct is greatly constricted in its lower portion and dilated above this region.

to the right shoulder. Vomiting and inability to retain any food became the main symptoms. Constipation developed after the onset of illness.

Emaciation was marked. There was an area of tenderness in the epigastrium extending into the right hypochondrium. The liver edge was palpable. Return from an enema was black. Serology and urinalysis were negative. The loss of weight was 30 pounds. Fluoroscopy revealed a dilated atonic stomach filling the entire abdomen and a stenotic duodenal lesion. A test meal could not be done since the slightest irritation caused vomiting.

The course was steadily and rapidly downward. Vomiting was the most prominent symptom; no type of food could be retained. The temperature was septic. Prostration was extreme and death occurred in coma. The entire duration from the onset was approximately one year.

Anatomical diagnoses: primary supraampullary carcinoma of the duodenum; acute suppurative inflammation of the upper retroperitoneal tissues; chronic interstitial pancreatitis; acute bronchopneumonia.

The entire first portion of the duodenum from 1 cm. below the pyloric ring to just above the papilla of Vater was a thick rigid tube with a very narrow lumen. Dense adhesions bound it to the pancreas. The mucosal surface was ulcerated, the cut surface hard and white. The papilla of Vater projected below the lower edge. The lower half of the common bile duct was tightly constricted, barely admitting a filiform bougie; its surface appeared normal. The pancreatic duct could not be probed.

Histology.—The ulcerated surface was formed by muscle and covered by a purulent exudate. The entire thickness of the duodenum in this region was infiltrated by neoplastic cells showing marked anaplasia, numerous mitoses and some gland formation. Both edges had a very atypical gland formation, suggesting strongly the glands seen at the edge of chronic

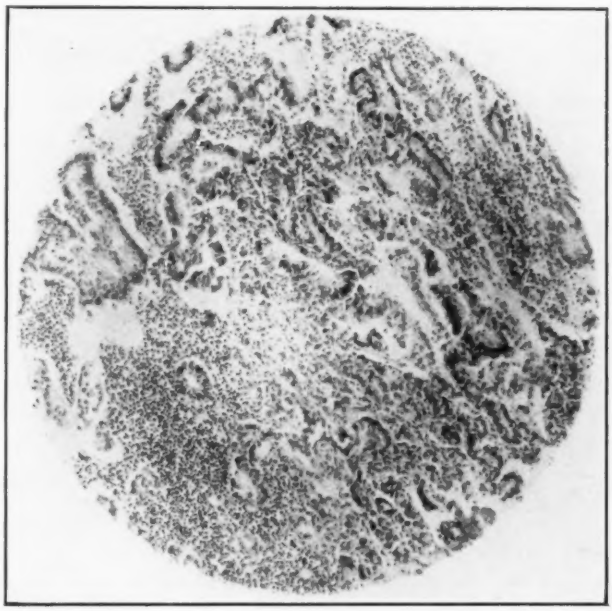

Fig. 4.—Case 2. Photomicrograph of the edge of the carcinoma showing the atypical gland formation.

ulcer. The constricted portion of the common duct was completely encircled by malignancy. The epithelium of duct and papilla was normal. Malignant invasion of the pancreas was present in portions of the perilobular stroma, normal ducts and malignant nests lying side by side in sharp contrast. Miliary abscesses and a diffuse streptococcal inflammation involved the upper duodenum and the retroduodenal and peripancreatic tissues.

DISCUSSION

The two cases present many of the characteristic features of duodenal carcinoma. In the first case, the hepatic complication was the predominent factor, a finding which coincides with all cases in which the primary site is the same. The late onset of jaundice suggests strongly that the papilla was involved by extension of a carcinoma originating in the duodenal mucosa of the neighborhood.

In the supraampullary carcinoma the vomiting was greater than usually occurs in pyloric malignancy. Occult blood in the stool and development of constipation associated with intense vomiting are stated to be of great value in differentiating this particular site of malignancy from that of the pylorus. The roentgenologic findings were of the greatest diagnostic importance.

The absence of metastases in both cases well exemplifies their late occurrence in duodenal carcinoma.

SUMMARY

The recent literature of duodenal carcinoma is summarized. Two cases are reported, one involving the periampullary, the other the supraampullary portion. Their salient clinical and anatomical features are given.

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ACCIDENTAL INJURY TO THE BOWEL DURING INTRAPERITONEAL INJECTION*

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THE peritoneal route of administration is utilized widely in the laboratory and clinic, especially for supplying fluids in dehydration and by pediatricians as a substitute for the intravenous route. Also important, as Pearcy and Weaver[3] pointed out, is the extensive intraperitoneal use of hypnotics by veterinary surgeons, investigators for anesthesia in laboratory animals, and more recently, for hypnosis in man (McGuire[2] and Thursz[5]). The paucity of evidence of injury to the abdominal viscera recorded during the development of peritoneal administration permits injection with relative impunity, providing a few simple precautions are regarded. Ravenel,[4] however, considered mechanical injury to the bowel the cause of fatal acute generalized peritonitis in a child, following two perforations of the jejunum after intraperitoneal injection of salt solution.

Sporadic erosions of obscure etiology in the bowel of cats and dogs anesthetized by nembutal intraperitoneally were observed recently by A. D. Keller.† He suggested that the bowel wall may have been injured by the injection needle or nembutal and demonstrated local tissue damage by this barbiturate. In view of this and the importance of peritoneal absorption, it seemed desirable to accumulate as much data as possible on the occurrence of injury to the bowel by the puncture needle and the changes produced by the more common barbiturate, saline, and glucose solutions when injected into the wall of the stomach and intestine. If injury may occur, precautionary measures should be emphasized. In addition, the accidental injection of a substance into the lumen of the stomach or bowel, instead of the peritoneal cavity, might account for the occasional absence of the usual response to the substance.

ACCIDENTAL INJURIES TO THE BOWEL

The stomach and bowel of approximately 150 dogs and 100 cats were carefully examined after periods of from ten minutes to three months following routine intraperitoneal injection of hypnotics for anesthesia. The ensuing six lesions found in the bowel were directly attributable to accidental injury:

1. A dog examined twenty minutes after nembutal injection demonstrated a linear dark gray red hematoma, 1 cm. by 0.3 cm., beneath the serosa on the lateral aspect of the large bowel near its midportion (Fig. 1, *E*). At one end of this was a needle-puncture wound of the serosa from which fresh blood was expressed.

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Received for publication, February 26, 1934.

†Personal communication.

2. In another dog given nembutal and examined an hour after injection, there was a large, recent intramural hematoma in the distal portion of the jejunum with beginning necrosis of the mucosa.

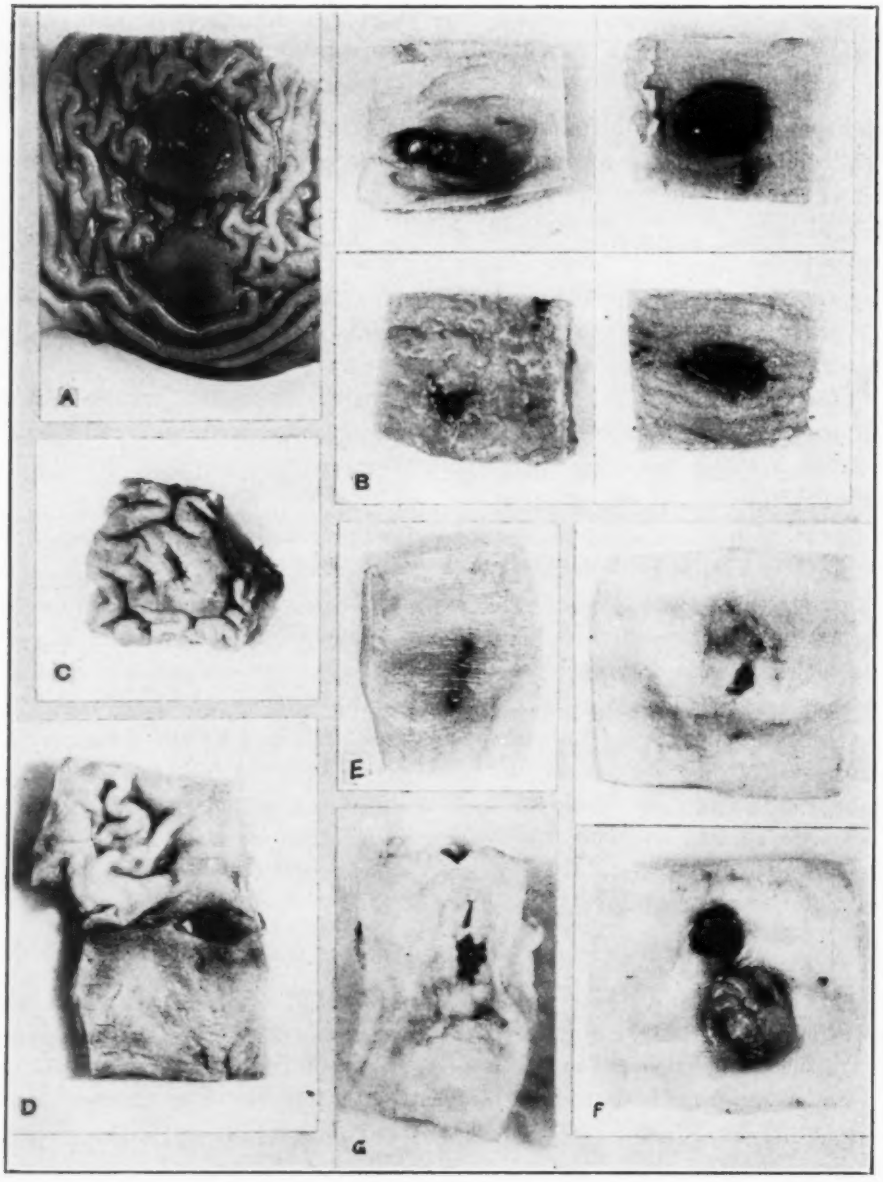

Fig. 1.—Morphologic changes in the stomach and bowel, the result of intramural injections of barbituric acid derivatives. *A*, marked edema and hyperemia of the cardiac portion of the stomach ten minutes after two 0.25 c.c. intramuscular injections of nembutal (1 gr. in 1 c.c.); *B*, erosions of the mucosa opposite subserosal hematomas of the jejunum one and nine hours after subserosal injections of 0.25 c.c. of nembutal (1 gr. in 1 c.c.); *C*, healing ulcer of the cardiac portions of the stomach three days after submucosal injection of 0.25 c.c. of amytal (10 per cent); *D*, large perforation of the duodenum, which occurred on the fifth day after intramuscular injection of 1 c.c. of dial (10 per cent); *E*, *F*, and *G*, injuries to the bowel produced by accidental intramural injections of nembutal (1 gr. in 1 c.c.) during routine intraperitoneal administration of this barbiturate for anesthesia (see text).

3. Six hours after receiving avertin, one dog demonstrated a circumscribed gray blue region in the serosa of the large bowel, 1.2 cm. in diameter, 10 cm. distal to the ileocecal valve. In the center there was a needle hole in the serosa, and the mucosa corresponding to the region was gray red and scraped away easily.

4. Dog 56-B was anesthetized by nembutal intraperitoneally five days before he was killed. There was a healing punched-out ulcer of the upper portion of the ileum (Fig. 1, *F*), 0.7 by 0.3 by 0.3 cm., with a moderately indurated and elevated margin from 2 to 4 mm. wide. Outside the defect in the lining was a gray pink organizing subserosal hematoma, 1.6 cm. in diameter, elevated 0.3 cm., the center of which was depressed and perforation of the ulcer had been prevented alone by the muscularis mucosae (checked by histologic examination).

5. Cat 63 died of acute generalized peritonitis, following a large perforation of the ileum (Fig. 1, *G*), 12 cm. proximal to the cecum, eight days after intraperitoneal nembutal for anesthesia.

6. Dog 67 was examined thirty-five days after receiving nembutal intraperitoneally. There was a stellate scar in the mesentery of the small bowel, 9 cm. from the cecum, that held in close apposition the adjacent serosal surfaces of a loop of ileum.

Experimental Procedure.—Through a small midline laparotomy incision under ether anesthesia and aseptic precautions, 155 subserosal, intramuscular, and submucosal injections of from 0.25 to 1 c.c. of the following substances were made with a sharp 26-gauge needle into the wall of the stomach and various levels of the small bowel of dogs: nembutal (pentobarbital, Abbott), 1 gr. in 1 c.c., as recommended for intraperitoneal use; nembutal, made up 1 gr. in 1 c.c. of sterile distilled water; dial (diallyl-barbituric acid, Ciba), 10 per cent with 40 per cent urethane and monoethylurea, as supplied for parenteral administration; 10 per cent solutions of sodium barbital and amytal; 5 and 50 per cent glucose; 0.9, 2, and 30 per cent sodium chloride solutions and sterile distilled water. The viscera were manipulated gently without clamps and multiple needle-puncture wounds were made into and through the wall without injection. The animals were killed and examined immediately after periods of from ten minutes to fifteen days.

Results.—The changes produced by the barbiturates varied according to the amount injected, the time elapsed between injection and examination, and according to the position of the substance within the wall. Those produced by dial were slightly more marked than the other derivatives studied. There were no apparent differences in degree of injury produced or healing processes among the stomach and various levels of the small bowel. Within a few minutes after injection (Fig. 1, *A* and *B*), the mucosa covering the thickened portion of the wall was hyperemic, thin, and soft. The vesicles formed by subserosal injections became hemorrhagic in about ten minutes, and the regions of necrosis in the lining over these were larger, but less marked, than those following submucosal and intramuscular injections. With-

in from twenty-four to forty-eight hours there were definite punched-out defects in the mucosa varying in size from a few millimeters (0.25 c.c.) to 1.5 cm. (1 c.c. injection) in diameter. The smaller ulcers were of the lining while the larger frequently extended as far as the serosa. One of these in the duodenum near the pyloric ring, produced by 1 c.c. of dial, perforated on the fifth day (Fig. 1, *E*). There was considerable edema and slight hyperemia in the zone of reaction about the recent ulcers and moderate induration during healing, which began in two or three days (Fig. 1, *C*) and was usually complete without scar of the lining, except following large ulcers, within eight to fifteen days. The subserosal hematomas organized and contracted and were ultimately absorbed. When produced by the larger subserosal injections, their centers occasionally became soft, and they healed with small scars of the serosa.

Distilled water, 0.9 per cent sodium chloride, and 5 per cent glucose produced thickening and slight hyperemia of the wall, which persisted but a few hours without noteworthy change of the lining. Small defects of the mucosa occasionally followed the larger injections of 2 per cent sodium chloride and 50 per cent glucose. The changes produced by 30 per cent sodium chloride were very similar to those of the barbiturates. Needle-puncture wounds of the wall, without injection, and punctures into the lumen were entirely healed and could not be discerned two days after operation.

Histologically, the early changes were characterized by extravasation of blood and partial or complete necrosis of the tissues at the site of injection. The muscularis mucosae retained its structure, unless a large intramuscular injection had been made, and evidently prevented more frequent penetration of the ulcers. During the development of an ulcer, there was little or no noteworthy reaction about the zone of necrosis. After two or three days, the extravasated blood began organization; fibroblastic tissue, slightly infiltrated with round cells, extended into it from all sides and regeneration of the mucosa had begun from the periphery of the lining defect. In five or six days there remained, as evidence of slight injuries, only small contracted, rather loose fibrous regions in the muscularis, over which the lining was narrow and devoid of folds. After small subserosal injections, organizing hematomas alone indicated the sites of injection. Tissue defects extending through the muscularis mucosae healed by granulation and ultimately produced small puckered scars of the mucosa.

DISCUSSION

During routine peritoneal administration to animals, injections often are made without due precaution and thorough postmortem examination is usually confined to parts significant only to a particular experiment. In view of this and the marked tendency for gastrointestinal lesions to heal, it seems possible that injury to the stomach and bowel or intraintestinal injection may occur more frequently than is generally appreciated. The same may be true in man during peritoneal administration of fluids and drugs, as pointed out by

Ravenel. More frequent serious injury to the bowel, after its wall has been injected, is probably prevented by loss of a portion of the injected substance through the wound in the serosa. It is significant that four of the six instances of inadvertent injury to the bowel recorded here occurred in the more fixed portion, where it is not free to shift position.

The lesions described here as being due to accidental injury to the bowel are not coincidental, in view of Ivy's[1] conclusion that gastrointestinal changes of any consequence occur rarely, if at all, in the dog. The degree of organization in the hematomas produced by the accidental injury to the bowel was proportionate to the time elapsed since intraperitoneal injection, and the more recent had needle-puncture wounds of the serosa.

The changes in the stomach and bowel following intramural injection of barbiturates may be due to alkalinity and slow absorption. The compact structure of the walls, however, probably plays a part by preventing dissemination. In the concentrations used, the hypnotics were isotonic with from 1.58 to 1.97 per cent sodium chloride. The freezing point of the dial solution could not be determined because of its association with other substances. These equivalent concentrations of salt solutions were slowly absorbed when injected yet produced only slight changes compared to those of the hypnotics and no changes of the lining suggesting necrosis by pressure.

Intraperitoneal injections should be made through a short-beveled medium-sized needle equipped with a stylus. The abdominal wall should be relaxed so that it may be picked up between the thumb and index finger and the thrust of the needle made obliquely to the plane of the wall. After penetration, the needle may be withdrawn slightly to insure freedom of its tip. Particular care should be exercised if the stomach or bowel is distended.

SUMMARY

1. Six accidental injuries to the bowel in cats and dogs following intraperitoneal injection are reported. One of these animals died of acute generalized peritonitis, the result of perforation.

2. The bowel is punctured or intraintestinal injection is made more frequently during intraperitoneal administration than is generally appreciated. Simple needle-puncture wounds of the stomach and small bowel heal without consequence.

3. The barbituric acid hypnotics, when injected into the wall of the stomach and small bowel, rapidly produce necrosis of the lining and subsequent changes of the deeper tissues. These changes are probably due to alkalinity and slow absorption of the substance. Although there is a distinct tendency for the lesions to heal, perforation of the wall may occur.

4. Glucose and sodium chloride solutions, unless concentrated, produce no damage when small amounts are injected into the wall of the stomach or small bowel.

5. It is not intended to discourage intraperitoneal administration but such injections must be made carefully to insure peritoneal absorption and freedom from injury to the gastrointestinal tract.

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THE EFFECT OF BACTERIA ON INSULIN*

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A FEW definitely established properties of insulin may be briefly summarized as an introduction to the present investigation:

Insulin in its purest crystalline form behaves like an amphoteric electrolyte, has an isoelectric point about pH 5.3, and shows a light absorption in the ultraviolet at exactly the same place as most proteins. It is coagulable by heat and yields simple amino acids on hydrolysis. Its physiologic activity is destroyed by proteolytic enzymes. According to Sjögren and Svedberg[1] it has a molecular weight of 35,100, comparable to that of a large number of proteins.

While the above properties strongly suggest that insulin is a protein, there is a possibility that the physiologically active principle might be a much smaller molecule adsorbed on crystalline material. There appear certain data not entirely in harmony with our conception of protein behavior. In his studies on crystalline insulin, Scott[2] reports:

"It has been observed by various workers that many diabetics who show localized sensitivity reactions to injections of beef insulin can be given hog insulin and show no such sensitivity reactions. Further, certain patients who show a local reaction to commercial preparations of beef insulin show similar reactions to beef crystalline insulin.[3] If crystalline insulin is a chemical entity, it is surprising that individuals should be hypersensitive to it—a protein substance already present in the body."

Those familiar with bacterial metabolism will recall that certain bacterial species are capable of splitting pure proteins and that others lack this faculty. *Proteus vulgaris* is an example of the former and *Escherichia coli* of the latter type. The present investigation endeavors to utilize these phenomena in further understanding the nature of insulin.

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Received for publication, February 24, 1934.

This investigation was supported in part by a grant from Eli Lilly and Company, Indianapolis, Ind.

Before entering into details about the experimental work a few words might be necessary regarding the following:

Organisms.—Pure cultures of *Proteus vulgaris* and *E. coli* (K-12) were employed.

Medium.—Several media were investigated, and the one finally selected to yield suitable results contains the following ingredients:

M/5 Potassium acid phosphate	50.0 c.c.
M/5 Sodium hydroxide	23.6 c.c.
Sodium chloride	5.0 gm.
Glucose	2.0 gm.

0.5 c.c. of a 1 per cent solution of the chlorides of magnesium calcium, and iron.

The above ingredients were dissolved and made up to a volume of 1 liter in a volumetric flask by the addition of distilled water that had been boiled and cooled to room temperature. If the ingredients are accurately measured the final pH is exactly 6.8.

Sterilization.—This was performed by filtration through a Chamberland candle L 3 and not by autoclaving.

Insulin.—Several preparations were investigated, but the majority of the experiments were performed on Lilly's product U-100 and on a sample of crystalline insulin secured from Abel's laboratory.

Test for Insulin.—The physiologic activity of insulin was determined roughly on rabbits. The strength of the crystalline material was considered to be 25 units per 1 mg. of the dry material. The tests that were carried out in this laboratory were performed simply to gain a rough estimate of the physiologic activity of the material subjected to the action of bacteria.

Procedure.—In performing our experiments great care was taken to insure sterility before inoculation. The medium is first introduced into sterile flasks, followed by the desired amount of insulin, and then inoculation. The flasks were then carefully rotated to obtain a thorough mixture of the ingredients. Samples were removed by means of sterile pipettes for the following tests: pH, sugar determination, bacterial count, and the physiologic assay of insulin introduced. Samples removed after one or more days of incubation (for the physiologic assay of insulin only) were treated with 2 or 3 drops of a 5 per cent phenol solution and allowed to stand for fifteen minutes at room temperature before injecting them into the animals. In this connection it is important to point out that the removal of bacteria by filtration through a Chamberland candle L 3 is not suitable, for the amount of insulin employed is small to begin with, and it was found by preliminary experiments that in such small amounts a considerable loss occurs. Apparently it is adsorbed on the candle.

Temperature.—In our earlier experiments incubation of the samples was carried out at a temperature of 37° C. This permitted a rapid growth of the organisms. This temperature, however, was not found suitable for our pur-

poses as insulin without bacteria incubated in weak solutions at 37° C. was found to lose its physiologic activity very rapidly.

Experimental.—Into two flasks containing 50 c.c. of the medium, 1 c.c. of U-100 was introduced into each. After three days' incubation at 37° C., 3 c.c. samples were removed of each flask and injected into two rabbits, respectively. The results are given in Table I.

TABLE I

	MG. BLOOD SUGAR PER 100 C.C.	
	INITIAL	ONE HOUR
Rabbit No. 1	119	105
Rabbit No. 2	103	100

Hydrogen Ion Concentration.—The most suitable pH of the medium was also investigated. Media containing the same ingredients as described earlier but varying in pH values from 6 to 6.8 were tried. The one most suitable for our purposes was found to be pH 6.8. As an illustration the following data are reported:

Each sample contained 25 c.c. of the medium and 60 units of insulin. The number of bacteria introduced into each was 16,000 per 1 c.c.

SAMPLE	pH	NUMBER OF BACTERIA AFTER THREE DAYS' INCUBATION AT 20° C.
1	6.0	45,000,000
2	6.2	50,000,000
3	6.4	80,000,000
4	6.8	84,000,000

Action of E. Coli (K-12) on Insulin.—In the following experiment both crystalline and amorphous insulin were used. Incubation was carried out in an incubator regulated at 20° C. The duration of the experiment was thirty-one days. In repeating this experiment for confirmation of the results reported in Table I, the time was cut down to ten days and fourteen days, respectively. This was necessary as the crystalline material at hand was very limited. The volume of the medium in the flasks was 100 c.c. to which 250 units of Lilly's U-100 and 250 units of crystalline insulin, respectively, were added. This experiment was controlled by inoculating 100 c.c. of the medium containing no added nitrogen with the same number of organisms. With no added nitrogen, it is obvious that any definite increase in numbers must arise from associated impurities in the medium constituents or from some extraneous source, such as atmospheric ammonia.

Proteus Vulgaris and Insulin.—Into two flasks containing 100 c.c. of the medium 200 and 100 units of Lilly's insulin were introduced and inoculated with *Proteus vulgaris.* The flasks were then incubated at 20° C. As the number of microorganisms growing from day to day is of relative importance, it is omitted from Table III, but it may be stated that the number introduced was 50,000 per 1 c.c. at the beginning of the experiment, and at the end of the

TABLE II

B. COLI AND INSULIN

Each sample contains 250 units of insulin per 100 c.c. of medium. Glucose in the medium is given in terms of mg. per 100 c.c. In performing the assay of insulin, each rabbit was subcutaneously injected with 1 c.c. (2.5 units) per kg. Blood sugar was estimated before and 1.5 hours after the injections.

INSULIN PREPARATION	TIME	MEDIUM			MG. BLOOD SUGAR PER 100 C.C.		
		SUGAR CONCENTRATION	pH	NUMBER OF B. COLI PER 1 C.C.	0 HR.	1.5 HR.	
	days	mg.			mg.	mg.	
Crystal	0	186	6.7	90,000	95	49	
Lilly's	0	187	6.8	90,000	91	65	
Control	0		6.8	90,000			
Crystal	1	188	6.7	81,000	88	31	convulsions
Lilly's	1	190	6.8	100,000	95	56	
Control	1			100,000			
Crystal	2	190	6.7	500,000	104	30	
Lilly's	2	190	6.8	300,000	90	30	
Crystal	3	183	6.7	8,000,000	115	53	
Lilly's	3	186	6.8	6,000,000	100	51	
Control	3			3,000,000			
Crystal	4	185	6.7	5,400,000	85		convulsions
Lilly's	4	180	6.7	7,500,000	87		convulsions
Crystal	5	184	6.7	5,400,000	No test		
Lilly's	5	174	6.7	8,200,000	No test		
Control	5			5,000,000			
Crystal	7		6.6	5,300,000	85	25	
Lilly's	7		6.6	80,000,000	80	33	
Control	7			3,500,000			
Crystal	8	180			89		convulsions
Lilly's	8	136		200,000,000	87	42	
Control	8			3,200,000			
Lilly's	10	110	6.0		90	60	
Crystal	12	180	6.6		95		convulsions
Lilly's	12	70	6.0		91		convulsions
Crystal	15			12,500,000			convulsions
Lilly's	15			320,000,000			convulsions
Crystal	22				84*	42	
Lilly's	22				92*	52	
Crystal	31				90*	40	

*The blood sugar of rabbits that received only 2 units of insulin per kg.

eighth day it grew to 50,000,000. That at the end of the eighth day the physiologic activity of the samples was almost completely destroyed can be seen from the data on blood sugar.

CONCLUSION

The *Proteus vulgaris* experiment indicates very clearly that this proteolytic microorganism has the faculty of completely destroying the physiologic activity of insulin.

TABLE III

Assay of insulin subjected to the action of *Proteus vulgaris*. Sample No. 1 contains 200 units of insulin and No. 2, 100 units. The amount of insulin administered to the animals was 2.5 units per kg.

SAMPLE NUMBER	INCUBATION PERIOD (DAYS)	SUBCUTANEOUS INJECTIONS (PER KG.)	MG. OF BLOOD SUGAR PER 100 C.C.	
			(0 HOUR) MG.	(1.5 HR.) MG.
1	0	2.5	91	37
1	1	2.5	83	45
1	3	2.5	87	convulsions
2	3	2.5	84	50
1	6	2.5	90	convulsions
2	7	2.5	93	95
1	8	4.0	87	88
2	8	4.0	91	93

Dealing with a microorganism that cannot attack a pure molecule of protein, the experiment on *E. coli* and insulin justifies the following conclusions:

1. *E. coli* has no effect whatever on the physiologic activity of insulin whether or not impure or crystalline insulin is subjected to its metabolic activities.

2. *E. coli* introduced into a medium in which the only source of nitrogen is derived either from insulin or from the impurities associated with it must derive its nitrogen requirements from the latter and not from the nitrogen of insulin.

3. When crystalline insulin is substituted for the commercial product, the growth of *E. coli* parallels its growth in the medium that does not contain insulin—and in which no other source of nitrogen is available. The slight difference encountered in bacterial count in the crystalline insulin experiment and in the control experiment (eight millions as compared to five millions in the control) may either be due to bacterial variation or to slight impurities associated with the crystals. Such nitrogen impurities adsorbed on the large molecule of insulin might have been derived from the ammonia used in bringing about crystallization (see Abel's method[4]). While such traces of nitrogen may not be noticeable in chemical analytical methods, they are detectable by microorganisms. Consequently, the evidence presented in this investigation supports the theory that insulin is a protein.

Further investigations on the action of other microorganisms on insulin are in progress.

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THE EFFECT OF MUCIN AND MUCINOIDS ON PEPTIC DIGESTION*

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THE following experimental data may prove of interest to those wishing to evaluate the behavior of gastric mucin and similar substances in terms of properties upon which therapeutic use might be predicated. No attempt will be made to review the extensive literature which has appeared since the original suggestion of Fogelson[1] and of Kim and Ivy[2] in 1931.

Commercial gastric mucin, kindly furnished us by the Wilson Laboratory, loses on dialysis as much as 18 per cent of its solids, of which a third is free tyrosine. A 5 per cent solution dialyzed has a viscosity of about forty times that of water. It is not precipitated by acids and is indeed difficult to precipitate by any of the reagents used for removal of native proteins which

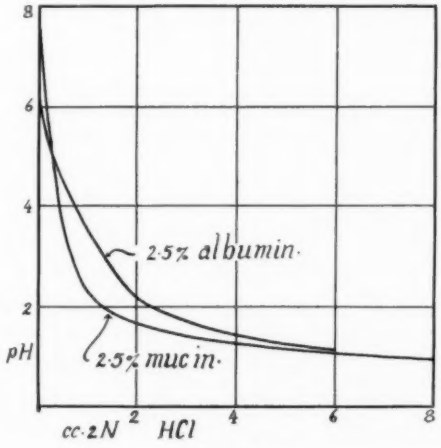

Chart 1.

will at the same time permit the larger cleavage fragments to remain in solution. Alcohol and alcoholic zinc sulphate are fairly satisfactory precipitants. Mucin is not appreciably digested by pepsin as might be anticipated from its history. It has about the same buffer value against acid as any common protein. Chart 1 shows that it is somewhat less effective than egg albumen of the same concentration.

In the following experiments we have studied the effects of mucin on the peptic cleavage of raw fibrin. This protein was selected as the substratum of choice because in the active ulcer, the layer of exudate-fibrin laid down on the surface is believed to be the necessary factor for organization and tissue growth which constitutes healing. Anything which will delay or prevent the digestive removal of this pellicle of fibrin should therefore be of potential

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Received for publication, February 16, 1934.

value in permitting normal healing to proceed uninterrupted. The figures in Table I and subsequent tables represent the net gain in soluble N in 25 c.c. of the filtrate, or 8.3 c.c. of the digest.

Fibrin and Mucin.—Raw fibrin was washed white, ground fine, and pressed free from gross moisture. Twenty grams of this material was used (dry weight 4.2 gm.) suspended in water or 5 per cent mucin, the pH adjusted to the desired level. Five cubic centimeters of 5 per cent pepsin was added, and the digest made up to 200 c.c. and sampled at once. Digestion was carried on at room temperature; the 25 c.c. samples taken were precipitated with 50 c.c. 2.5 per cent $ZnCl_2$ in 95 per cent alcohol. Digestion was determined by Kjeldahl's method on 25 c.c. aliquots of the filtrate. Table I shows a typical digestion series.

TABLE I

DIGEST	pH	NET GAIN—C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
Fibrin + Pepsin HCl	0.6	0.4	0.8	1.4	2.7	6.5	8.3
Fibrin + Pepsin HCl + Mucin	0.6	0.0	0.1	0.3	0.7	3.5	8.0
Fibrin + Pepsin HCl	1.2	1.4	3.2	4.9	5.4	5.4	5.4
Fibrin + Pepsin HCl + Mucin	1.2	0.5	0.9	1.6	2.7	5.1	5.5
Fibrin + Pepsin HCl	2.0	1.4	2.5	3.6	4.8	5.0	5.1
Fibrin + Pepsin HCl + Mucin	2.0	0.5	0.8	1.2	1.9	3.3	3.6
Fibrin + Pepsin HCl	3.0	0.0	0.0	0.1	0.4	0.7	1.6
Fibrin + Pepsin HCl + Mucin	3.0	0.0	0.0	0.2	0.4	0.9	1.5

The precipitation of primary fragments by the alcoholic zinc precipitant is influenced by the acidity of the particular sample taken. The more acid the digest, the more nitrogen remains in solution. The distortion of the series as a whole, however, does not in any way affect the interpretation of data obtained at the same pH levels. Throughout the range studied, from pH 0.3 to pH 3.0, mucin is found to retard the digestion of fibrin. During the first period of from two to five hours it may exceed a 50 per cent reduction. By the end of from one to three days, however, the results are identical. The action of mucin therefore is equivalent to reducing the concentration of the active enzyme. The inhibition is one of rate only. It is most striking where peptic digestion is most rapid. At pH 3 digestion is slow, and mucin has no apparent effect.

Mucin and Other Proteins.—The inhibitory effect of mucin is not specific for raw fibrin. We have digested casein, coagulated albumin, fibrin coagulated by heat and by alcohol with and without mucin present. The results are similar to those illustrated above. A typical example is given in Table II.

Snail Mucin.—Snail mucin, kindly furnished us by the Burroughs Wellcome Laboratory, has very different properties from the gastric mucin. It is slowly dispersed in slightly alkaline solutions and is precipitated by acids. It is digested by pepsin sufficiently so that interpretation of results is difficult. The enzyme evidently divides itself between the mucin and the fibrin, but, since the digestion of the mixture is not a summation, we may assume some inhibition of fibrin digestion. In view of the flocculation of mucin in acids and its

digestibility, it would appear to be of less value as a protective agent than gastric mucin, which retains its physical properties in acid, and does not digest. (See Table III.)

TABLE II

CASEIN AND MUCIN

DIGEST	pH	NET GAIN IN C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
Casein + Pepsin HCl	1.0	5.0	6.2	8.4	12.0	14.8	17.6
Casein + Pepsin HCl + Mucin	1.0	1.1	0.8	2.2	4.2	6.7	10.1
Casein + Pepsin HCl	2.0	2.2	4.5	6.4	8.9	11.5	12.3
Casein + Pepsin HCl + Mucin	2.0	0.3	1.4	2.2	4.2	7.3	8.9
Casein + Pepsin HCl	3.0	1.1	1.7	3.1	3.9	6.7	8.4
Casein + Pepsin HCl + Mucin	3.0	0.0	0.3	0.6	1.4	4.5	7.8

TABLE III

EFFECT OF SNAIL MUCIN

DIGEST	pH	NET GAIN IN C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
Fibrin + Pepsin HCl	1.04	0.8	1.5	2.2	3.8	5.6	8.0
Fibrin + Pepsin HCl + Mucin	1.08	0.3	1.0	1.6	2.9	5.7	7.6
Mucin + Pepsin HCl	1.00	0.7	1.0	1.2	--	2.6	3.1
Fibrin + Pepsin HCl	1.95	0.8	1.2	2.0	3.0	4.7	6.6
Fibrin + Pepsin HCl + Mucin	1.94	0.6	1.0	1.5	2.7	6.0	7.3
Mucin + Pepsin HCl	2.0	0.6	0.7	1.0	--	2.4	2.9
Fibrin + Pepsin HCl	3.06	0.2	0.2	0.3	0.7	1.6	2.5
Fibrin + Pepsin HCl + Mucin	3.08	0.4	0.6	0.7	1.0	2.3	3.5
Mucin + Pepsin HCl	3.00	0.5	0.5	0.6	--	1.5	2.1

TABLE IV

FIBRIN AND CHONDROITIN SULPHATE, 1 PER CENT

DIGEST	pH	NET GAIN IN C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
SAMPLES DILUTED 1:2 WITH WATER							
Fibrin + Pepsin HCl	0.99	1.8	3.1	4.6	6.5	7.6	7.7
Fibrin + Pepsin HCl + Chond.	1.03	0.0	0.2	0.8	2.0	4.3	5.9
SAMPLES DILUTED 1:2 WITH TRICHLORACETIC ACID							
Fibrin + Pepsin HCl	1.03	0.4	0.8	1.6	3.2	5.6	7.6
Fibrin + Pepsin HCl + Chond.	1.08	0.1	0.3	0.7	1.9	4.7	6.0

TABLE V

THE EFFECT OF VEGETABLE MUCINOIDS

DIGEST	NET GAIN IN C.C. N/5 N IN FILTRATE				
	pH	1 HR.	5 HR.	1 DAY	3 DAYS
Fibrin + Pepsin HCl	1.0	3.4	8.0	9.9	9.8
Fibrin + Pepsin HCl + Okra	1.0	1.0	5.1	10.9	11.9
Fibrin + Pepsin HCl + Agar	1.0	3.0	6.6	7.9	8.6
Fibrin + Pepsin HCl + Starch	1.0	1.8	5.4	8.9	9.1
Fibrin + Pepsin HCl + Gum Ghatti	1.0	2.3	5.3	10.5	10.4

Chondroitin Sulphate.—Babkin[3] has pointed out that gastric mucin may contain some free chondroitin or mucoitin sulphate and that this compound exerts a retarding effect on the digestion of coagulated egg albumen in Mett's

tubes after twenty-four-hour exposure. We have repeated this experiment using raw fibrin and confirm this observation. In this experiment, too, we were able to differentiate between the dispersion of the fibrin, and its cleavage into nonprecipitable fragments. In one digest the samples were precipitated with 5 per cent trichloracetic acid in water; in the other, the samples were merely diluted. The inhibitory effect of the chondroitin sulphate is much more correctly represented by the digest where no precipitant was used. Solution of fibrin goes on much more rapidly than cleavage to nonprecipitable fragments in the control digests of fibrin alone. The presence of chondroitin sulphate retards dispersion much more than it does further cleavage, as may be seen in Table IV. Dispersion and cleavage in the presence of chondroitin sulphate are practically identical. This fact is of particular significance because it indicates that mucin itself is probably far more potent in delaying solution of fibrin by pepsin than it is in retarding its further cleavage, and from the point of view of therapy this is the important effect.

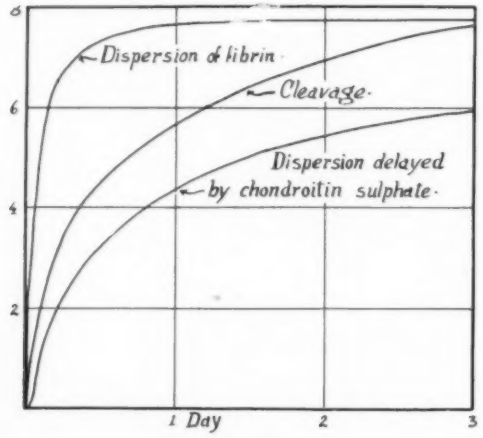

Chart 2.

Okra, Agar-Agar, Gum Ghatti, and Starch.—Dispersions of these materials were made to roughly approximate the physical properties of 5 per cent gastric mucin. Five per cent trichloracetic acid in 95 per cent alcohol was used as precipitant, two volumes to one of the sample. A typical series is given in Table V.

Of these vegetable mucinoids the okra dispersion most nearly resembles mucin in physical properties and in the retardation of peptic activity.

SUMMARY

The experiments indicate a temporary delay in the digestive removal of fibrin covered and protected by such mucinous materials as gastric mucin and okra. This inhibition has the same effect as reducing the concentration or activity of pepsin in the digesting mixture. Any delay in the removal of the fibrin pellicle covering the ulcer surface until the stomach contents pass out would appear to be of real significance in preventing interruption of the healing process.

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THE TOXIC EFFECTS OF SULPHUR ON GUINEA PIGS AND RABBITS*

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WE DEMONSTRATED the inhibitory effect of sulphur, even in concentration as small as 1:40,000, on the growth of tubercle bacilli on culture media as a scientific exhibit at the Southern Medical Association Nov. 14 to 17, 1933. As a preliminary to animal experimentation we began a study of the effects of sulphur on guinea pigs and rabbits.

We first did a series of intracardiac injections in guinea pigs and intravenous injections in rabbits with 1 per cent colloidal sulphur in distilled water. (This suspension was not a perfect colloidal solution.) Large doses of this solution were used, ranging from 5 to 10 c.c. both in rabbits and guinea pigs.

There were several fatal reactions at the time of injections, which we thought were due to emboli. These reactions occurred in only three animals. Nine rabbits were used for intravenous injections. These rabbits received 5 c.c. of 1 per cent colloidal sulphur in distilled water per pound of body weight for each injection. Several of the rabbits died at the time of the second injection; but most of them survived four such doses of sulphur and were then killed and autopsied on the fourth and sixth days from the time of the first injection.

The autopsy results varied slightly in minor details, but the almost uniform results were:

1. The lungs showed areas of interstitial pneumonia of a chemical type, without round cell infiltration, but with a few hemorrhagic areas.

2. There were many typical infarctions throughout the lungs and, in some instances, they appeared to be numerous enough to have caused death, whereas in others there were only two or three such areas.

3. The kidneys showed a marked nephritis characterized by marked swelling of the tubules in some and a marked necrosis of the renal cortices in others.

4. The liver sections showed many sulphur crystals throughout the tissue and a few small areas of degeneration.

*Received for publication, March 29, 1934.

5. Sections of the heart and spleen showed no miscroscopic changes.

One rabbit received 150 c.c. of 0.031 per cent filtered colloidal sulphur, intravenously over a period of five days. No ill-effects were observed, and he is now living and well, six months after the time of injection.

After intraspinal injections of rabbits with light suspensions of sulphur dissolved in oil (1:500), the rabbits survived and showed no pathologic condition of the cord after twenty-four hours. Injections of a heavy suspension of sulphur in oil (1:55) caused death of the animal in twelve hours. Postmortem examination showed an edema of the cord and deposits of sulphur crystals around the base of the brain (two rabbits used).

One to two grams of sulphur were injected into the peritoneum of rabbits through a small cannula. It produced a marked necrosis of the abdominal wall with dense adhesions and necrosis of the gut about the point of injection.

Intraperitoneal injections of 10 c.c. of 1 per cent colloidal sulphur in guinea pigs produced a knotting of the omentum incapsulating the sulphur without other adhesions being formed. Injection of 2 c.c. of 1:500 sulphur in oil produced the same result. (Five guinea pigs and four rabbits were used for intraperitoneal injections of sulphur.)

A series of 28 guinea pigs and 2 rabbits were confined to boxes 36 inches long, 18 inches wide and 22 inches deep with open tops. Three or 4 animals were confined to each box and a continuous sulphur duster was installed, delivering a fine sulphur suspension which could only be detected with a strong beam of light. For this we used the commercial three-hundred-mesh sulphur and colloidal sulphur prepared by the Heyden Chemical Corporation. In these experiments the results did not vary markedly with either of the types of sulphur used.

To our surprise, all the rabbits died in from two to four days. The stomach walls were perforated and absolutely necrotic. There was some congestion of the liver, but, this did not seem to be very marked. The guinea pigs all died in from one to three days, and presented the same pathologic picture as did the rabbits. As the material had been blown over the animals as well as over their food and bedding, there was no way to determine accurately just how much of the sulphur had been ingested.

We then tried a series of 41 guinea pigs and 2 rabbits giving doses of from 2 gm. to 0.50 gm. of sulphur per kilogram of body weight, directly into the stomach through a small stomach tube, using in some the colloidal sulphur and in others the flowers of sulphur. To our surprise a similar condition developed, though much more rapidly.

All the animals in this last series died within from two to twenty-four hours. The stomach walls were necrotic, kidneys, intensely congested, and liver slightly so. The spleens showed little change as did the hearts and lungs. This was not a postmortem change, for the same picture was observed when several of the animals were examined under anesthesia.

The kidneys on microscopic sections showed an intense necrosis, almost as though the kidney had come into direct contact with a very powerful

chemical poison. The stomachs were too necrotic to recover more than the connective tissue stroma for microscopic sections.

A possible explanation of these findings was suggested in that sulphur acids may be produced in animals unable to vomit as are the guinea pigs and rabbits. This, however, may have no bearing, but it was thought the very rapid devastating effect of ingested sulphur on the tissues of these animals might be of interest.

CONCLUSIONS

Sulphur when ingested by guinea pigs and rabbits produces a necrosis of the stomach and a severe nephritis.

LABORATORY METHODS

HEMOGRAPHY IN DIAGNOSIS, PROGNOSIS, AND TREATMENT BASED ON 6,000 SCHILLING HEMOGRAMS*

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OF THE methods now used for differentiation of the white blood cell count, experience has shown the system developed by Schilling in 1911 to be the most practical and scientific. It takes but little more time than the ordinary differential, and is easily learned. Most subsequent investigators have agreed that Schilling has classified the cells properly according to age and defensive qualities. The importance of the additional information gained by the Schilling method of analyzing the blood picture is rapidly receiving the recognition of clinicians and hematologists. Interpretation of the various types of hemograms becomes apparent after a little experience in using this method.

We have used the modification of the Schilling method of constructing and analyzing hemograms in over 6,000 blood studies of various infections and intoxications which we call "hemography." This includes the recording of daily hemograms, if possible, arranging them in series so that the various components may be compared easily during the course of the disease. At the close of the case the serial hemograms, united by the "shiftograph," form a complete chart of the blood studies or a "shiftogram."

As the use of hemography has developed the importance of the "Schilling index" has been emphasized and the "shiftograph," "multiple index" and "lethal index" have been added. These facilitate the interpretation and comparison of hemograms.

Only a brief description of the shift cells and segmenters in a hemogram can be offered at this time.

Myelocytes.—Nucleus; large and oval, round, or notched. When the notch is present, it is not more than 25 per cent of the diameter of the nucleus. The structure is filaceous and spongy in appearance, and may or may not contain nucleoli. It stains bluish violet to dark reddish blue. Cytoplasm; relatively narrow and stains light blue, although this color is masked by evenly distributed neutrophilic granules which give the cytoplasm a yellowish brown appearance, except where the granules have been thinned out by toxic degeneration.

Juveniles.—Nucleus; deeply indented or U-shaped. The notch extends 50 per cent or more of the diameter of the nucleus. Nucleoli may be present in

*From the Division of Clinical Pathology, Laboratories, Philadelphia General Hospital.
Received for publication, January 20, 1934.

the ends. It stains light reddish violet or bluish red and has a wide, flat, lacy background containing early chromatin patches. The depth of the notch distinguishes it from the myelocyte. The cytoplasm is the same as in the myelocyte.

Stabs.—(Degenerated or pathologic.) Nucleus; narrow, cylindrical, and bent like the letters or numbers U, V, S, W, T, E, 6, 8, 3. Segmentation may be almost complete but the lobes are connected by bands of chromatin wider than a thread. The margins are uneven and the chromatin is gathered in irregular dark compact masses between which sharply defined light apertures appear. The nucleus is pyknotic, twisted, and contorted and takes a reddish purple or dark blue stain. When the chromatin is removed in extreme toxic degeneration the nucleus is light in color and filaceous in structure but may be distinguished from the juvenile by its narrowness. The term "band form," which implies

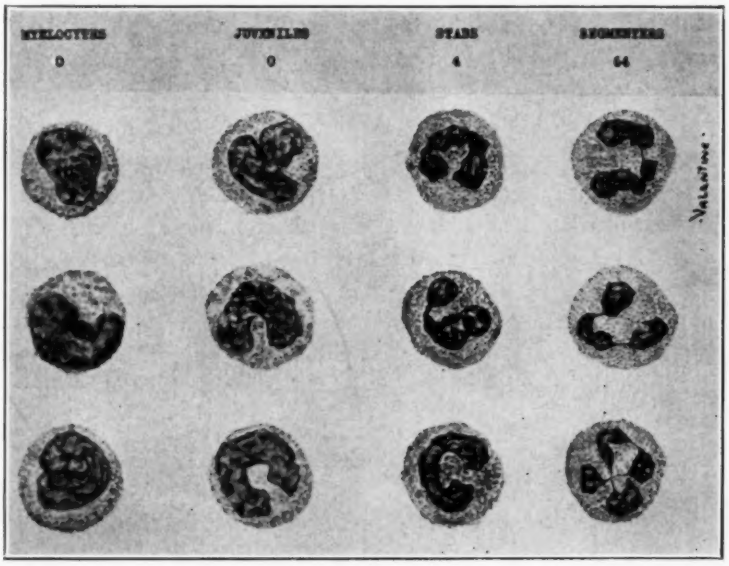

Fig. 1.—Staining with Wright's stain and buffer solution. (1) Wright's stain one minute. Cover the slide with stain, 10 to 15 drops. (Cells fixed by the methyl alcohol and staining begun.) (2) Buffer solution three minutes. Add an equal quantity of buffer solution to the Wright's stain on the slide, 20 to 30 drops. (Staining completed.) (3) Wash in distilled water. (4) Blot. (5) Decolorize in 95 per cent alcohol one second to remove stain deposit. (6) Wash in distilled water immediately until the slide stops streaking. This must be done quickly to stop the decolorizing action of the alcohol. (7) Blot.

Wright's stain, Biosol Products Co., Rochester N. Y., 1 tablet to 10 c.c. of absolute methyl alcohol C.P.

Buffer solution pH 6.45.
Stock Sol. I. 28 c.c.
Stock Sol. II. 72 c.c.
Dist. H_2O 900 c.c.

Stock Sol. I. $Na_2HPO_4 \cdot 2H_2O$ (11.87 grams dissolved in 1,000 c.c. distilled water) $Na_2HPO_4 \cdot 2H_2O$ is obtained by spreading $Na_2HPO_4 \cdot 12H_2O$ in a thin layer and drying in the air for two weeks.

Stock Sol. II. M/15 KH_2PO_4. (9.077 grams dissolved in 1,000 c.c. distilled water.)

flatness, has been applied to this nucleus, but the term "stab," or "staff," more correctly describes its cylindrical character. The cytoplasm is the same as in the myelocyte.

Segmenters.—Nucleus; is divided into several distinct segments, usually from two to five. These either show no visible connections, or are joined to-

gether by thin threads of chromatin. The distinguishing feature between the segmenters and lobulated stabs is the width of the chromatin joining the lobes. That of the segmenter is never wider than a fine thread. The lobes of the segmenter are oval and have the same cylindrical quality of depth presented by the stab nucleus. The chromatin is compact and may be divided into fairly distinct fields of oxy- and basi-chromatin, which stains dark reddish purple to dark blue. The cytoplasm is the same as that of the myelocyte.

Description of Hemogram.—The hemogram is an outline of horizontally arranged figures representing the components of the blood picture. Various combinations signify different conditions, and changes in these combinations from day to day constitute the characteristics by which we may determine improvement, retrogression or complication of the case.

"Schillingizing" the neutrophiles, or subdividing them into four classes according to their age and defensive strength determines the quality of the cellular army. With the body at physiologic rest the normal peripheral blood contains no myelocytes, no juveniles, 4 stabs and 64 segmenters; a total of 68 neutrophiles in each 100 white cells of all types. An increase in the shift cells, i.e., myelocytes, juveniles and stabs with a corresponding decrease in segmenters is called a "left shift" and means stimulation or irritation. Conversely a decrease in shift cells with a simultaneous increase in segmenters constitutes a "right shift," and signifies lessened toxicity. The type of left shift is termed degenerative when stabs are increased, regenerative when stabs and juveniles alone, or as many as four myelocytes appear; and leukemoid when stabs, juveniles and five or more myelocytes appear in the hemogram. The type of shift indicates the intensity of the intoxication by its qualitative extent to the left.

The "Schilling index" is the ratio of the combined shift cells to segmenters.

M + J + St : Seg

0 0 4 : 64 4 to 64, or a normal Schilling index of $\frac{1}{16}$.

This index gives the proportion of inefficient phagocytes to efficient phagocytes. The higher the index, the greater the quantitative shift and the more severe the intoxication.

The "multiple index" is the Schilling index multiplied by 16. Thus the normal is one. It has the same significance as the Schilling index but gives information in whole numbers, showing the number of times a given shift is greater than normal.

The "lethal index" gives the ratio of myelocytes to segmenters, or if no myelocytes are present, the ratio of one-half the juveniles to segmenters. This index gives the proportion of weakest to strongest phagocytes. When the lethal index approaches one, it suggests a diagnosis of septicemia and a prognosis of fatality.*

*In attempting to prognose the date and time of expiration we are using the following table with a fair degree of accuracy:

Lethal index 1 and multiple index 100, death in 48 hours or less.
Lethal index 1 and multiple index 200, death in 24 hours or less.
Lethal index 1 and multiple index 300, death in 12 hours or less.
Lethal index 2 or over, regardless of multiple index, death in 12 hours or less.

The "shiftograph" is a vertical graph of the Schilling index arbitrarily adopted by us to express the relationship of the quantitative shift to the qualita-

Diagnosis	Hemoglobin Hb	Mils RBC RBC	Total WBC WBC	Lethal index LI	Multiple Index MI	Schilli Index ShI	Myelocytes M	Juveniles J	Sticks St	Segmenters Sg	Tot neutrophiles TN	Lymphoblasts Lb	Large Lymphs L	Interned Lymphs I	Small Lymphs S	Total Lymphs TL	Monocytes M	Eosinophiles E	Basophiles B	Toxic Granules TG	Inclusion bodies IB	Hegon power RP	Neut per cu mm Neut 5000	Threshold T	Segmenters cu mm Sgs 4500	Type of left shift Tp Norm
	100	5.0	7500	0	1	1/16	0	0	4	64	68	0	5	1	20	26	4	2	0	0	0	0				
												TYPE 1.														
Rup. Abdom.aortic aneurysm	50	2.7	10400	0	8	1/2	0	0	28	58	86	0	4	0	8	12	2	0	0	0	0	0	Str	15624	SlIp	10672	Deg
Traumatic ruptured kidney	75	3.4	16300	0	11	2/3	0	0	28	42	70	0	4	0	16	20	10	0	0	0	0	0	Str	11084	SlIp	6520	Deg
Ruptured ectopic pregnancy	40	2.5	18500	0	16	1.	0	0	36	36	72	0	0	0	26	26	2	0	0	0	0	0	Str	13320	MdIp	6660	Deg
Bleeding duodenal ulcer	85	4.3	22100	0	8	1/2	0	0	32	64	96	0	0	0	4	4	0	0	0	0	0	0	Str	21316	SlIp	14144	Deg
Sarcoma of stomach-infected	80	3.4	22700	0	15	1.	0	0	36	34	70	0	0	0	26	26	2	2	0	0	0	0	Str	15890	MdIp	7718	Deg
Incarcerated hernia	90	4.1	20800	0	26	1/6	0	0	14	83	97	0	3	0	0	3	0	0	0	0	0	0	Str	20176	SlIp	17264	Deg
												TYPE 2.														
Puerperal sepsis	75	3.2	25900	1/8	78	4.9	2	8	68	16	94	0	2	0	2	4	2	0	0	40	10	Str	24252	SvIp	4128	Deg
Lobar pneumonia	80	4.0	20900	2/3	104	6.5	3	19	52	12	90	0	4	0	6	10	0	0	0	20	5	Str	18720	SvIp	2496	Leuk
Ruptured appendix-peritonitis	90	4.4	17600	0	48	3.0	0	2	66	22	90	0	4	0	4	8	2	0	0	25	6	Str	15840	SvIp	2376	Deg
Intestinal obstruction	85	4.2	39900	1/12	48	3.0	2	6	64	24	96	0	0	0	2	2	2	0	0	25	5	Str	38408	SvIp	9552	Deg
Chronic nephritis-uremia,terminal.	75	3.1	38700	1.	176	11.0	9	10	62	7	97	0	2	0	5	7	13	0	0	12	3	Str	33804	SvIp	2706	Leuk
Scarlet fever	90	4.7	23900	1/11	8	1/2	4	2	22	44	72	0	6	0	14	20	0	0	0	20	5	Str	19436	MdIp	10472	Leuk
Erysipelas	90	4.1	10600	1/5	48	3.0	4	6	42	18	70	0	2	0	28	30	0	0	0	0	0	Str	7420	SvIp	1908	Deg
Gastritis-peritonitis,iodine-lysol	35	4.5	8000	1/2	80	5.0	6	4	50	12	72	0	4	0	20	24	4	0	0	30	8	Str	5760	SvIp	1920	Leuk
Carbon monoxide poison	100	5.1	49700	1/20	40	2.5	0	2	52	20	74	0	6	0	10	16	10	0	0	0	0	Str	36778	SvIp	9940	Deg
												TYPE 3.														
Strangulated hernia-peritonitis	75	3.6	4800	1/3	208	13.0	2	20	58	6	86	0	2	4	8	14	0	0	0	30	10	SvIp	4128	SvIp	258	Deg
Mesenteric thrombosis-peritonitis	100	5.0	4800	8.	604	38.0	8	4	26	0	38	0	16	0	42	58	2	0	0	50	20	Par	1744	Par	0000	Leuk
Lobar pneumonia	80	4.3	3100	3.	256	16.0	12	22	30	4	68	2	12	0	4	18	14	0	0	25	5	SvIp	2108	SvIp	124	Leuk
Osteomyelitis	75	3.4	4900	3.	132	12.0	18	16	42	6	82	0	4	2	10	16	2	0	0	40	10	SvIp	4018	SvIp	294	Leuk
Lobar pneumonia	50	2.5	1400	40.	960	60.0	40	10	10	0	60	0	0	0	40	40	0	0	0	50	20	Par	840	Par	0000	Leuk
												TYPE 4.														
Subacute bact. endocarditis	75	3.6	6700	0	2	1/7	0	0	6	40	46	0	8	0	40	48	6	0	0	0	0	SlIp	3082	SlIp	268.	Deg
Abscessed teeth	95	5.1	9900	1/35	4	1/4	0	2	8	38	48	0	16	0	18	34	16	2	0	0	0	SlIp	4272	SlIp	2562	Deg
Trichiniasis	90	4.6	7700	1/5	32	2.0	0	4	17	11	32	0	13	0	18	31	1	36	0	0	0	MdIp	2464	SvIp	647	Deg
Catarrhal appendicitis			11150	0	10	2/3	0	0	26	36	62	0	8	0	22	30	2	6	0	0	0	Str	6882	MdIp	3976	Deg
Rheumatic arthritis			9300	0	8	1/2	0	0	16	30	46	0	6	0	48	54	0	0	0	0	0	SlIp	4590	MdIp	2940	Deg
Subac.exacerb.of chronic appendicitis			7600	0	12	3/4	0	0	18	24	42	0	15	0	34	49	3	6	0	0	0	MdIp	3192	SlIp	1824	Deg
Massive empyema-draining	50	2.5	8700	0	22	1.4	0	0	46	32	78	0	8	0	14	22	0	0	0	0	0	Str	6786	MdIp	2784	Deg
												TYPE 5A.														
Pertussis			65000	0	48	3.0	0	0	30	10	40	4	2	0	54	60	0	0	0	0	0	Str	26000	SvIp	6500	Deg
Gumma of liver	80	4.0	5400	0	32	2.0	0	0	40	17	57	0	0	0	37	37	3	3	0	0	0	MdIp	3538	SvIp	918	Deg
Chronic hyperplastic appendicitis			5200	0	8	1/2	0	0	24	50	74	0	6	0	20	26	0	0	0	0	0	MdIp	3848	MdIp	2600	Deg
												TYPE 5B.														
Typhoid fever	80	4.0	6600	0	48	3.0	0	2	16	6	24	0	10	0	58	68	3	0	0	0	0	SvIp	1584	SvIp	396	Deg
Influenza			4700	0	48	3.0	0	6	24	10	40	0	18	0	30	48	12	0	0	0	0	SvIp	1880	SvIp	470	Deg
Agranulocytosis (symptomatic)			4000	3	64	4.0	3	1	0	1	5	0	14	28	45	95	0	0	0	0	0	Par	200	Par	40	Deg

Fig. 2.—Types of hemograms. Key: Sl, slight; Md, moderate; Sv, severe; Dp, depression; Str, strong; Deg, degenerative; Reg, regenerative; Leuk, leukemoid; Par, paralyzed.

tive shift. It extends left with the left shift as the index increases and vice versa. The shiftograph is placed in such relation to the columns of neutrophiles that the quantity of the shift at any angle of the graph may be compared with the quality of the shift or the types of cells in the various columns at that point.

Reproductive Power "R.P." and Threshold "T."—It is generally stated that in mild infections maturation of the neutrophiles in the bone marrow keeps pace with the demand for cells, producing a leukocytosis of the adult type; that in severe infections, although there may be a leukocytosis, the demand for mature cells is greater than the bone marrow can supply, and immature cells appear in the peripheral blood; while in overwhelming infections either production or maturation or both may break down, producing a regenerative or leukemoid shift with or without leukocytosis. Again certain irritants or toxins may fail to stimulate the bone marrow or even inhibit production and maturation, then we find leukopenia with immature cells in the peripheral blood.

Whether the factor or factors responsible for these changes in the bone marrow are stimulants or irritants, or whether the changes are the result of positive or negative chemotaxis, we are not prepared to say definitely. For convenience we simply use the term "reproductive power," "RP" of the bone marrow as its capacity for quantitatively producing neutrophiles for the cellular army of defense. It is an index of the reserve strength of the patient and is estimated in general by the total white count and in particular by the percentage of neutrophiles and the number of neutrophiles per cubic millimeter. And we use the term "threshold" "T," as that mechanism which qualitatively controls the maturity of the cells that pass from the bone marrow into the peripheral blood, a normal threshold discharging mature neutrophiles only (64 per cent segmenters and 4 per cent normal stabs), a depressed threshold permitting immature cells to escape, the degree of left shift varying with the degree of depression, and it is with this understanding that these terms will be used throughout this paper in interpreting the size (quantity) and maturity (quality) of the cellular army.

The lymphocytes are Schillingized routinely as the lymphatic shift is very significant. Lymphocytes are divided into four classes: lymphoblasts, large lymphocytes, intermediate lymphocytes, and small lymphocytes. As the large lymphocytes increase at the expense of the small, a left shift is designated, which points to an acute stimulation of the lymphatic tissue or an incipient immunohealing process. A right shift means an increase in the small lymphocytes with a decrease of the large and indicates a chronic stimulation or a definitely established immunohealing phase of repair and convalescence.*

TYPES OF HEMOGRAMS

Type 1.—Mild acute stimulation of noninfectious or mildly infectious agents, resulting in a slight left shift.†

*We realize, of course, that size alone is not an accurate criterion of age of lymphocytes, but this criterion has seemed valid in the shift encountered.

†The one notable exception to the mildly infectious or noninfectious character of conditions which give rise to a Type 1 hemogram is acute suppurative early gangrenous appendicitis with or without rupture of the appendix. The clinical symptoms must be typical of acute appendicitis. The features which characterize this exception are a high total W.B.C. count, 18,000 to 20,000, high neutrophilia, 80 to 100 per cent but only a slight or moderate left shift showing a Schilling index of 1 or less, and a multiple index of 16 or less. When the threshold fails to permit the toxic left shift to be expected with the high white blood cell count, high neutrophilia and typical appendicial symptoms, we call it a "frozen threshold." Toxin elaborated in the small area of gangrene may be responsible for the inactivity, fixing, or freezing of the threshold.

Blood Picture:	Leukocytosis	10,000 – 20,000	Sh. I.	¼ – 1
	Neutrophilia	80 – 90%	M. I.	4 – 16
	Lymphopenia	5 – 20%	L. I.	0 – ½
	Monopenia	0 – 4%		
	Eosinopenia	0 – 2%		

The reproductive power "RP" is strongly stimulated. The threshold is only slightly depressed. The left shift is slight or moderate, of the degenerative type, sometimes bordering on the regenerative. Noninfectious stimulants producing this picture are, violent exercise, menstruation, pregnancy, and parturition. Noninfectious but severe stimulants are, internal hemorrhage, ruptured ectopic pregnancy, ruptured aortic aneurysm, or ruptured vessel and hernias that are incarcerated but not strangulated. Cysts with twisted pedunceles, tumors that are not infected or tumors before necrosis has occurred cause this mild type of shift, later producing a more severe picture.

Infections producing this picture are due to organisms of low virulence or are superficial in nature so that the products of infection are not confined as superficial wounds affecting chiefly the epithelium or draining abscesses. It is also seen in infections affecting mucous membranes with little vascular reaction, as in acute catarrhal cystitis, gastritis, or appendicitis and in the so-called appendicosis.

Type 2.—Severe acute irritation, by virulent infections, cytotoxins or poisons, resulting in a marked left shift.

Blood Picture:	Leukocytosis	10,000 – 100,000	Sh. I.	1.5 – 20 plus
	Neutrophilia	90 – 100%	M. I.	20 – 300 plus
	Lymphopenia	0 – 10%	L. I.	¼ – 1 plus
	Monopenia	0 – 4%		
	Eosinopenia	0 – 1%		

"RP" is strong and severely irritated. Neutrophiles 8,000 to 90,000. "T" is strongly depressed and the number of segmenters is reduced; the left shift is regenerative to leukomoid.

This picture is characteristic of many acute infectious diseases, e.g., septicemia, the meningitides, pneumonia, and diphtheria. It is seen in acute suppurative inflammation of mucous and serous membranes with infiltration of the surrounding tissues or with necrosis and gangrene, e.g., in acute suppurative appendicitis, volvulus, intussusception, peritonitis, empyema, abscesses, or confined pus in any location. Certain poisons may produce this type of hemogram, e.g., arsenic, lead, mercury, phenol and its derivatives, and illuminating gas.

Type 3.—Lethal acute irritation by overwhelming infections, resulting in an extreme left shift, with a depressed regenerative power.

Blood Picture:	Leukopenia	5,000 – 1,000	Sh. I.	3 – 20 plus
	Neutrophilia	90 – 100%	M. I.	50 – 300 plus
	Lymphopenia	0 – 10%	L. I.	¼ – 1 plus
	Monopenia	0 – 2%		
	Eosinopenia	0 – 1%		

"RP" is inhibited or paralyzed; neutrophiles 5,000 to 500 or less. "T" is severely depressed or paralyzed; the shift is leukomoid in type. This hemo-

gram is produced by extremely virulent acute infection. It is usually seen in septicemia. When it follows a Type 2 hemogram it means bone marrow exhaustion and paralysis with a fatal prognosis. In these cases the total white count is not reliable, as an index of the degree of intoxication, for although the total white count is falling, the left shift is becoming more extreme. Observation of this fact definitely illustrates the fallacy of judging the severity of an infection by the ordinary white count and differential. At the same time it emphasizes the importance of the shift in making a hemogramic prognosis.

Type 4.—Subacute stimulations, infections and intoxications, resulting in a moderate left shift, or the lymphomonocytic phase of healing convalescence, the latter following Types 1, 2, and 3.

Blood Picture:	White blood cells	6,000 – 12,000	
	Neutrophiles	50 – 70%	Sh. I. less than one
	Lymphocytes	30 – 50%	M. I. less than 16
	Monocytes	0 – 10%	
	Eosinophiles	1 – 5%	

"RP" is strong, but shows a low grade irritation.

"T" is only slightly depressed. The shift is usually degenerative in type although a few myelocytes and juveniles may occur. The pronounced feature is a moderate to marked lymphocytosis with an occasional slight monocytic increase. This picture is typical of long-continued or lowgrade intoxication. It is also seen transitionally when an acute infection becomes chronic, or when a chronic infection shows a subacute exacerbation. It is common in a subsiding acute infection. As the leukocytosis and neutrophilia fall, the lymphocytes, monocytes, and eosinophiles increase and the shift goes to the right. It is then called the lymphatic or lymphomonocytic immunohealing phase of convalescence.

It is characteristic of lowgrade septicemia, subacute bacterial endocarditis, arthritis, subacute exacerbation of chronic appendicitis; foci of infection, i.e., abscessed teeth, old appendicial abscess or sinusitis; chronic empyema, pyelitis, draining lung abscess, and degenerating or metastasizing tumors. With an acute flare-up of this type of infection, e.g., extension of suppuration, failure to drain, etc., the blood picture changes to one of the more acute types.

Type 5.—Lymphatic type with a moderate left shift. This is caused by acute infections which inhibit or fail to stimulate granulopoiesis and chronic infections which stimulate lymphopoiesis.

Blood Picture:	Normal or leukopenia	10,000 – 1,000	Sh. I. ½ – 2 (Except pertussis)
	Neutrophilia	50% or below	M. I. 8 – 32
	Lymphocytosis	30% or above	
	Monocytosis	6 – 20%	
	Eosinopenia	0 – 2%	

"RP" is inhibited or not stimulated; neutrophiles average 3,500.

"T" is mildly depressed. The shift is usually degenerative or may border on the regenerative type with a few juveniles or occasional myelocyte. This picture is produced by:

(A) Low grade, chronic toxins which stimulate lymphopoietic tissue, including tuberculosis, syphilis, Basedow's disease and healing processes.

(B) Acute infections which inhibit or fail to stimulate the granulopoietic tissue, including measles, mumps, malaria (latent), influenza, and typhoid fever. It is also seen in subacute or chronic appendicitis, abdominal adhesions, and ovarian cysts. A sudden change from this lymphatic hemogram to a Type 2 or 3, means an acute exacerbation or complication, e.g., secondary pyogenic infection in tuberculosis; onset of pleurisy or pneumonia in influenza; otitis media or bronchopneumonia in measles; perforated ulcer and peritonitis or pyelonephritis, in typhoid fever; orchitis in mumps, etc.

A primary right shift, with a Schilling index of less than $\frac{1}{16}$ is seen occasionally in pernicious anemia, *Diphyllobothrium latum* infestation, carcinoma with cachexia and anemia, syphilis, pregnancy, malaria, and ankylostomiasis.

CASES ILLUSTRATING THE VARIOUS TYPES OF HEMOGRAMS

Type 1.—Incarcerated femoral hernia in a white female aged seventy-one years. There was nausea and vomiting, but no other signs of intestinal obstruction. The hemogram was made about eight hours before operation. We were asked whether the hernial contents had become strangulated. At operation a normal loop of gut was released and replaced in the abdominal cavity.

Hemography: Shiftogram is a Type 1 hemogram, with high leukocytosis, high neutrophilia, extreme lymphopenia, and a very slight left shift.

Hemogramic Diagnosis: Acute noninfectious irritation, incarcerated hernia without strangulation. (Clinically: incarcerated hernia?)

Hemogramic Prognosis: Good.

The condition was considered an acute stimulation, rather than an infectious process because of the high leukocytosis with 97 per cent neutrophiles, but only a mild degenerative shift to the left. The noninfectious nature of the irritation was shown by such a slight depression of the threshold that the segmenters per cubic millimeter almost equalled the total neutrophiles. The slight degenerative shift of 14 stabs was too few to indicate pus; the Schilling index of $\frac{1}{6}$ was very small and the multiple index only 2.6 times normal. Incarceration without strangulation was indicated by the very slight shift. Strangulation would have shown extreme depression of the threshold, with segmenters less than half the neutrophiles and a severe qualitative left shift, including myelocytes and juveniles. The Schilling index would have been greater than one, the multiple index greater than 16, and the lethal index small or large, depending on the severity of infection, gangrene of the intestine, or onset of peritonitis. The prognosis was good because there was no hemogramic evidence of actual injury to the intestines.

Type 2.—Lobar pneumonia in a white male, aged forty years. The first hemogram was made on the seventh day of the disease, about six hours before the crisis occurred. Following the crisis there was an apparent relapse, but the temperature again fell by crisis and remained normal to recovery.

Hemography: All the hemograms in this shiftogram except the last are Type 2. The hemogramic diagnosis and prognosis are taken from the first hemogram.

Hemogramic Diagnosis: Severe acute infection (Clinically: lobar pneumonia).

Hemogramic Prognosis: "Bad at present, unless at the time of crisis."

The diagnosis was justified by the extreme leukocytosis, high neutrophilia, leukomoid left shift, high indices, and the presence of Freyfeld's toxic granules and Doehle's inclusion bodies.

A conditional bad prognosis was given on the first hemogram, as it was taken six hours before the crisis (see discussion), and because there were only 6,096 segmenters in a total neutrophilic army of defense of 35,052. Thus, while the defensive force was large, it was of very poor quality. The severity of the infection was also indicated by the high Schilling, multiple, and lethal indices.

In tracing the course of the disease, the shiftograph pointed to a relapse with a wide left shift in Hemogram 3, followed by a continued right shift to convalescence and

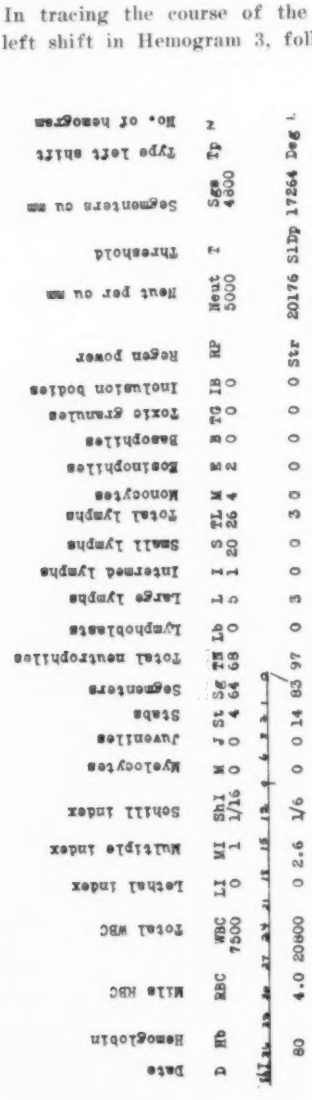

Fig. 3.—Shiftogram of incarcerated hernia illustrating a Type 1 hemogram.

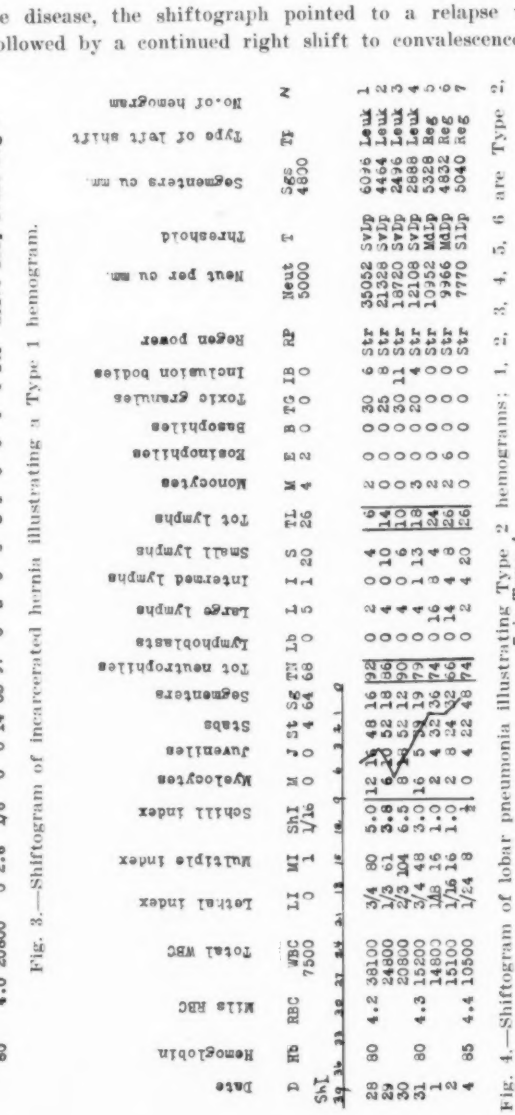

Fig. 4.—Shiftogram of lobar pneumonia illustrating Type 2 hemograms; 1, 2, 3, 4, 5, 6 are Type 2, 7 is Type 4.

recovery. The first attempt to enter the lymphatic phase of immunohealing was made in Hemogram 5, with a fall in neutrophiles, a rise in lymphocytes, and the disappearance of toxic granules and inclusion bodies. Full convalescence was shown by Hemogram 7, a Type 4, with a lymphatic right shift to small lymphocytes, a neutrophilic right shift to a multiple index of eight, and a high normal total white count.

Type 2.—Sepsis of unknown origin. The first hemogram was recorded twenty-five days following the onset of illness. Three weeks later, acting on a hemogramic diagnosis of confined pus, and the clinical evidence of continued sepsis and slightly enlarged tender liver,

a tentative diagnosis of subdiaphragmatic abscess was made and the abdomen opened. A subhepatic or duodenal abscess was suspected but complete exploration could not be done on account of the weakened condition of the patient. Death occurred forty-eight hours later.

Hemography: All the hemograms of this shiftogram are Type 2, the neutrophilic phase of defense. Diagnosis, prognosis, and treatment were suggested by the first hemogram and confirmed by subsequent blood pictures.

Fig. 5.—Shiftogram of abscesses of the liver, pyemia. All hemograms illustrate Type 2.

Hemogramic Diagnosis: Acute suppurative inflammation, probably abscess formation (clinically: sepsis of unknown origin).

Hemogramic Prognosis: Doubtful, dependent on the location of the suppuration and its treatment.

Treatment suggested: Establishment of free drainage.

This condition was called acute because of the high leukocytosis, neutrophilia and the regenerative left shift. The relatively high percentage of lymphocytes (20 per cent), associated with a high leukocytosis and a wide left shift indicated an infectious process of some duration and suggested an attempt at healing.

The course of the disease traced by the shiftograph, pointed to a complicating septicemia in Hemogram 4. Nonspecific immunotransfusion was recommended and given. This forced the shift strongly to the right within twenty-four hours. It then fluctuated slightly for the next few days, due probably to extension of the infection. Following laparotomy the shiftograph extended widely to the left, terminating in a severe leukemoid left shift with the death of the patient.

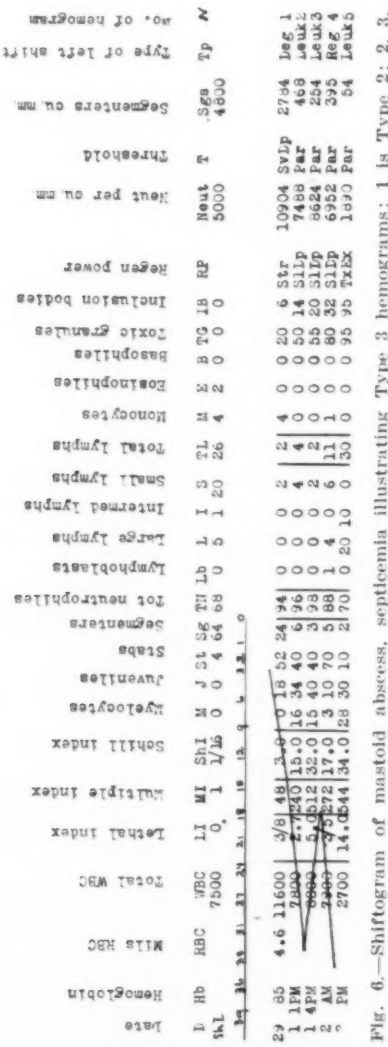

Fig. 6.—Shiftogram of mastoid abscess, septicemia illustrating Type 3 hemograms; 1 is Type 2; 2, 3, 4 and 5 are Type 3.

Type 3.—Acute mastoiditis in a white female aged twenty-one years. The first hemogram was made eighteen hours after the patient developed clinical signs suggestive of acute mastoiditis. A bilateral mastoidectomy was done the same day. After a short postoperative improvement acute septicemia developed and the patient died within two days.

Hemography: Although the first hemogram is of the second type, the next four show this to be a case of lethal acute irritation, a Type 3. The first count, taken just prior to operation, was interpreted as follows:

Hemogramic Diagnosis: Acute suppurative inflammation (clinically: mastoiditis).

Hemogramic Prognosis: Hopeful but depends on immediate drainage of the primary focus of infection.

Treatment suggested: Prompt establishment of free drainage.

The first hemogram showed a severe acute irritation by the presence of leukocytosis, high relative neutrophilia, extreme lymphopenia and the wide qualitative and quantitative left shift of the regenerative type. The prognosis was more grave because of the disproportion between the slight leukocytosis and the wide left shift.

The second hemogram was taken just following operation, and showed a transition to Type 3, the so-called lethal irritation, with a falling total white count, no higher than normal,

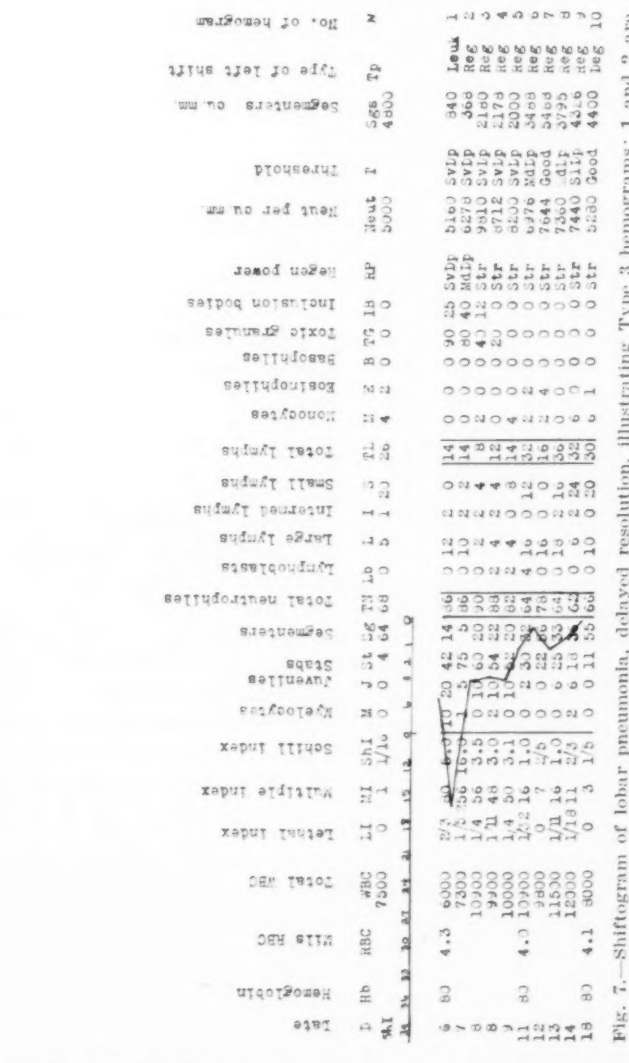

Fig. 7.—Shiftogram of lobar pneumonia, delayed resolution, illustrating Type 3 hemograms; 1 and 2 are Type 3; 3, 4, and 5 are Type 2; and 6, 7, 8, 9, and 10 are Type 4.

accompanied by a left shift becoming more severe. The interpretation of the second hemogram was as follows:

Hemogramic Diagnosis: Septicemia.

Hemogramic Prognosis: Fatal in twenty-four hours if the septicemia is not combated.

Treatment suggested: Nonspecific immunotransfusion.

The diagnosis of septicemia was justified by the falling white count, leukemoid left shift, and lethal index of over one. Following a Type 2 hemogram, we interpreted it as toxic exhaustion and bone marrow collapse with a fatal prognosis.

The treatment suggested could not be adopted and the patient died within the predicted twenty-four hours.

Type 3.—Bilateral lobar pneumonia in a white male, aged eighteen years. The first hemogram was taken on the day of admission, the sixth day of illness. On this day a pseudocrisis occurred; the temperature dropped to normal, but the pulse remained high and the patient looked very badly. On the seventh day, the patient's condition being even less hopeful, several small nonspecific immunotransfusions were given within the succeeding seventy-two hours. Recovery was uneventful.

Hemography: This shiftogram shows the first two hemograms to be a Type 3 lethal picture of toxic exhaustion, with a transition to three hemograms of the Type 2 neutrophilic phase of defense, and terminating with five Type 4 of healing and convalescence. The hemogramic diagnosis, prognosis and suggestion for treatment were based on the first hemogram.

Hemogramic Diagnosis: Acute infection, septicemia, no defense (clinically: lobar pneumonia).

Hemogramic Prognosis: If posterisis, fatal in forty-eight hours, unless immediate strenuous treatment is instituted.

Treatment suggested: Nonspecific immunotransfusions.

The condition was called acute infection because of the neutrophilia, lymphopenia, wide qualitative and quantitative left shift and high Schilling and multiple indices. Septicemia was suggested by the high percentage of toxic granules and inclusion bodies, with myelocytes almost equal to segmenters, giving a lethal index of $\frac{2}{3}$. No defense, or very low resistance, and toxic depression of the bone marrow was shown by the low total white count of 6,000, neutrophiles 5,160, and segmenters only 840, for protection against a pneumococcus septicemia. The conditional prognosis was justified by the severe depression of the threshold and regenerative power of the bone marrow, giving the defensive force a very poor quality. The Schilling index of 5, the multiple index of 80, the lethal index approaching one and nearly 100 per cent toxic granules pointed to an early termination, unless the toxic pressure was rapidly reduced. Nonspecific immunotransfusion was suggested as in our experience it had been the most effective treatment for septicemia.

The second hemogram is Type 3, but of less severity. It was taken after several small transfusions had been given. The quantitative shift had increased but the more important qualitative shift had turned sharply to the right. Myelocytes and juveniles had decreased markedly in number, while the stabs which are nearly as efficient as segmenters, had increased. While the shift to the right had occurred, without the usual increase in the number of segmenters, it was a qualitative right shift, nevertheless, and denoted improvement. The lethal index had decreased from $\frac{2}{3}$ to $\frac{1}{5}$.

Hemograms 3, 4, and 5 still showed the condition of the patient to be serious but improved. The neutrophilic phase of defense continued active with the neutrophilia and lymphopenia. The regenerative power was stronger with neutrophiles up to 8,000 per c.mm. The multiple index had dropped from 256 to 50. The myelocytes had practically disappeared, but the juveniles were still high. Hemogram 6 changed abruptly from Type 2 to Type 4, from the active neutrophilic phase of defense to the lymphatic immunohealing phase of convalescence. In percentage the neutrophiles were about normal, but a relative and absolute lymphocytosis with a lymphatic left shift occurred. Toxicity had been reduced or eliminated. The regenerative power and threshold were improved. The neutrophiles remained around 7,000 and the segmenters had risen to 4,000. All indices had been reduced, and the toxic granules and inclusion bodies had disappeared. Except for a temporary setback, the shiftograph showed a progressive right shift to convalescence and recovery of which Hemogram 10 is a typical example.

Type 4.—White male, aged forty-five years, with a history of lassitude, dullness, lack of energy, anorexia, and a slight loss of weight. He occasionally ran a slight afternoon elevation of temperature. The condition cleared up one week after an abscessed tooth had been extracted.

Hemography: Both hemograms of this shiftogram are Type 4, showing a high normal white count, neutropenia, lymphocytosis, monocytosis, and slight left shift.

Hemogramic Diagnosis: Subacute infection (teeth, tonsils or pharynx). (Clinically: possible focus of infection.)

Hemogramic Prognosis: Good.

Treatment suggested: Eliminate any foci of infection.

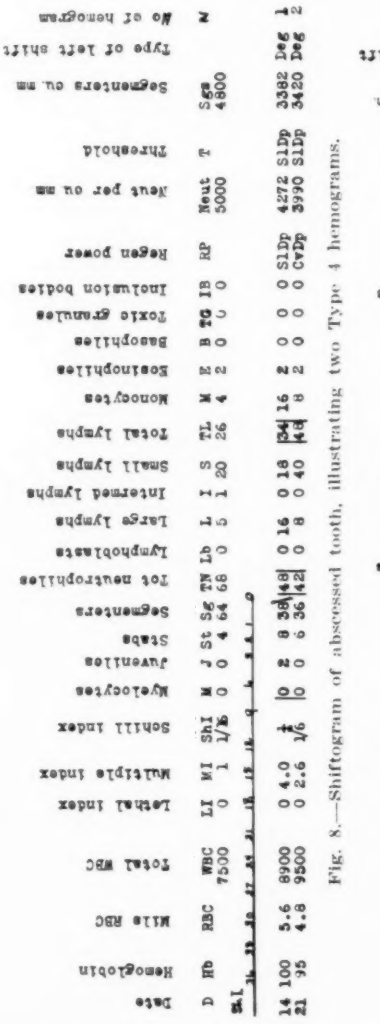

Fig. 8.—Shiftogram of abscessed tooth, illustrating two Type 4 hemograms.

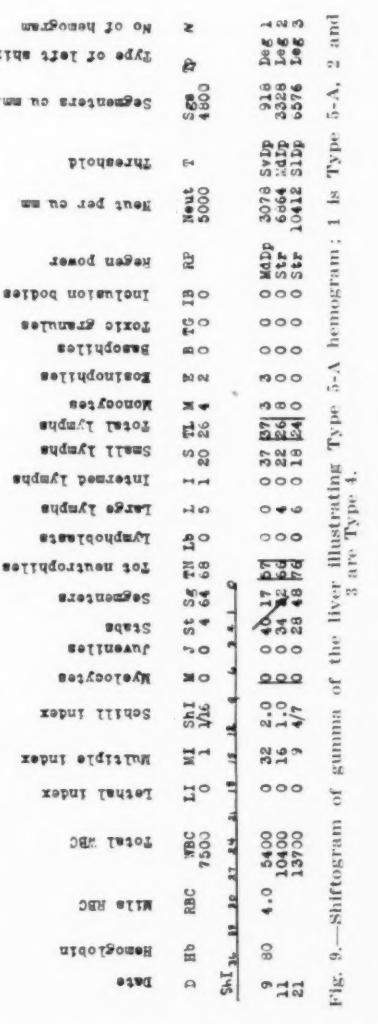
Date
Hemoglobin
Mills RBC
Total WBC
Lethal index
Multiple index
Sohlli index
Myelocytes
Juveniles
Stabs
Segmenters
Tot neutrophiles
Lymphoblasts
Large lymphs
Intermed lymphs
Small lymphs
Total lymphs
Monocytes
Eosinophiles
Basophiles
Toxic granules
Inolusion bodies
Regen power
Neut per cu mm
Threshold
Segmenters cu mm
Type of left shift
No of hemogram
9 80 4.0 5400 0 32 2.0 0 0 40 17 57 0 0 0 37 37 3 0 0 0 0 M4Dp 3078 SvDp 916 Deg 1
11 10400 0 16 1.0 0 0 34 32 66 0 4 0 22 26 8 0 0 0 0 Str 6664 M4Dp 3328 Leg 2
21 13700 0 9 4/7 0 0 28 48 76 0 6 0 18 24 0 0 0 0 0 Str 10412 S1Dp 6576 Leg 3

Fig. 9.—Shiftogram of gumma of the liver illustrating Type 5-A hemogram; 1 is Type 5-A, 2 and 3 are Type 4.

The first hemogram was made before the patient's focus of infection had been suspected. The diagnosis of subacute infection was made because of the slight left shift of 4 multiples, bordering on the regenerative type, as it included two juveniles. The monocytosis and lymphocytosis, with a left shift of lymphocytes to 16 of the large type, also pointed to the fairly acute lymphatic type of irritation which is seen in low grade infections.

The second hemogram was made one week after the abscessed tooth had been extracted. The total white count was about the same, but the neutrophiles had shifted to the right to 2.6 multiples, no juveniles being seen. The lymphocytes had increased and a lymphatic right shift had occurred. The number of small lymphocytes had doubled, indicating the lymphatic healing phase.

Type 5-A.—Gumma of the liver in a white female, aged thirty-nine years with pain in the right upper quadrant for several years. The patient's symptoms were gradually becoming worse; the liver was enlarged and tender. Blood Wassermann was positive. Operation disclosed the gumma of the liver, confirmed by biopsy. The patient improved with antisyphilitic therapy.

Hemography: The first hemogram of this shiftogram, on which the diagnosis was based, was a Type 5-A.

Hemogramic Diagnosis: Chronic suppurative hyperplastic inflammation (probably syphilis or tuberculosis). (Clinically: gumma.)

Hemogramic Prognosis: Poor.

Active inflammation was shown by the presence of more than 35 shift cells, a fairly wide degenerative shift with only 918 segmenters per c.mm. out of a total of 3,078 neutrophiles. Chronicity was shown by the lymphocytosis of 37 per cent, all of the small type. This was a lymphatic right shift and indicated efforts at healing. Hemograms 2 and 3 are Type 4. They followed the operation which disclosed gumma of the liver and showed the reaction of the patient to surgery; and the subsequent attempt at immunohealing.

Date	Hemoglobin	Mils RBC	Total WBC	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Tot neutrophiles	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Neut per cu mm	Threshold	Segmenters cu mm	Type of left shift	No of hemogram
D	Hb	RBC	WBC	LI	MI	ShI	M	J	St	Sg	TN	Lb	L	I	S	TL	M	E	B	TG	IB	RP	Neut	T	Sgs	Tp	N
			7500	0	1	1/16	0	0	4	64	68	0	5	1	20	26	4	2	0	0	0		5000		4800		
ShI	34	32	30	27	24	21	18	15	12	9	6	3	2	1	0												
7	80	4.0	7200	1/12	12	4/5	4	6	30	50	90	0	2	0	4	6	0	4	0	10	2	Str	6480	MdLp	3600	Reg	1
8			5500	0	19	1.2	0	0	24	20	44	0	52	0	0	52	0	4	0	50	14	SvLp	2420	SvLp	1100	Leg	2
9			5000	1/12	56	3.5	0	2	40	12	54	0	16	0	24	40	4	0	2	50	6	SvLp	2700	SvLp	600	Reg	3
10			5100	0	8	1/2	0	0	18	34	52	0	26	0	22	48	0	0	0	0	0	SvLp	2652	SvLp	1734	Leg	4
11			5000	0	8	1/2	0	0	24	46	70	0	6	0	18	24	2	4	0	0	0	SvLp	3500	SvLp	2300	Deg	5
13			4800	0	20	1.3	0	0	34	26	60	0	8	0	32	40	0	0	0	0	0	SvLp	2880	SvLp	1248	Deg	6
14	85	4.3	4500	0	17	1.1	0	3	30	30	63	0	3	0	30	33	0	1	3	0	0	SvLp	2835	SvLp	1350	Deg	7
15			5500	0	8	1/2	0	0	24	51	75	0	0	2	20	22	3	0	0	0	0	MdLp	4125	MdLp	2825	Deg	8

Fig. 10.—Shiftogram of influenza illustrating Type 5-B hemogram; 2, 3, 4, 6, and 7 are Type 5-B, 1 is a Type 2, and 5 and 8 are Type 4.

Type 5-B.—Influenza in a white male, aged twenty-eight years. The patient ran a typical course with headache, malaise, chills and fever, and cough. On January 10 patient was discharged apparently well but readmitted the following day with a return of symptoms. He was finally discharged in good health on January 15.

Hemography: All hemograms except the first, fifth and eighth on this shiftogram are Type 5-B, with a low normal white count, neutropenia lymphocytosis and a fairly wide left shift. The first hemogram is a Type 2, showing a high neutrophilia.

Hemogramic Diagnosis: Acute infection (clinically: influenza).

Hemogramic Prognosis: Good.

Acute infection was diagnosed from the first hemogram which showed the high neutrophilia and extreme lymphopenia common to all infections. Influenza was indicated by the second hemogram, which presented the low white count, neutropenia, lymphocytosis and lymphatic left shift characteristic of this disease when considered with the clinical symptoms.

In tracing the course of the disease the shiftograph showed a left shift peak on the third day, seen quite frequently in this condition, followed by a right shift apparently of a convalescent nature on the fifth day. A wide left shift on the sixth day, coinciding with the clinical relapse, was followed by a continuous right shift to a beginning convalescence on the ninth day.

DIFFERENTIAL DIAGNOSIS

The noninfectious, infectious or toxic nature of many conditions may be difficult to distinguish clinically. In these cases hemography may be of great

Diagnosis	Hb	RBC	WBC	LI	MI	Shi	M	J	St	Sg	TN	Lb	L	I	S	TL	M	E	B	TC	IB	RP	Neut per cu mm	Threshold	Segmenters cu mm	Type of left shift	Tp
	Hemoglobin	Mils RBC	Total WBC	Lethal index	Multiple index	Schilli index	Myelocytes	Juveniles	Stabs	Segmenters	Tot neutrophiles	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Neut per cu mm	Threshold	Segmenters cu mm	Type of left shift	Tp
A. Incarcerated hernia	80	4.0	20800	0	2.6	1/6	0	0	14	83	97	0	3	0	0	3	0	0	0	0	0	Str	20176	S1Dp	17264	Deg	
B. Strangulated hernia	80	4.2	24500	1/9	28.	1.8	4	4	56	54	98	2	0	0	0	2	0	2	0	100	40	Str	24020	SvDp	8330	Reg	
A. Ovarian cyst twisted on pedicle			19600	0	10.	2/3	0	0	20	58	78	0	2	0	14	16	6	0	0	0	0	Str	15288	S1Dp	11368	Deg	
B. Rup. appendicial abscess			17600	0	48.	3.0	0	2	66	22	90	0	4	0	4	8	2	0	0	25	6	Str	15840	SvDp	3872	Reg	
A. Tumor of lung			12000	0	4.	1/4	0	0	16	64	80	0	12	0	2	14	2	4	0	0	0	Str	9600	S1Dp	7680	Deg	
B. Costo-phrenic abscess			30700	0	40.	2.5	0	0	54	22	76	0	6	0	0	6	14	0	2	0	0	Str	23946	SvDp	6754	Deg	

Fig. 11.—Hemograms showing differential diagnosis of (A) noninfectious and (B) infectious processes.

assistance to the clinician for infectious processes with similar clinical pictures, falling into the same type of hemogram, are difficult to differentiate hemograph-

ically, while those falling into different hemogramic types are easily differentiated. The following three comparisons are used for illustration:

(A) is a Type 1, and (B) a Type 2 hemogram in each case. They both show a high leukocytosis, high neutrophilia, and marked lymphopenia.

Fig. 12.—Hemograms showing differential diagnosis of processes that may be similar clinically but of different hemogramic types.

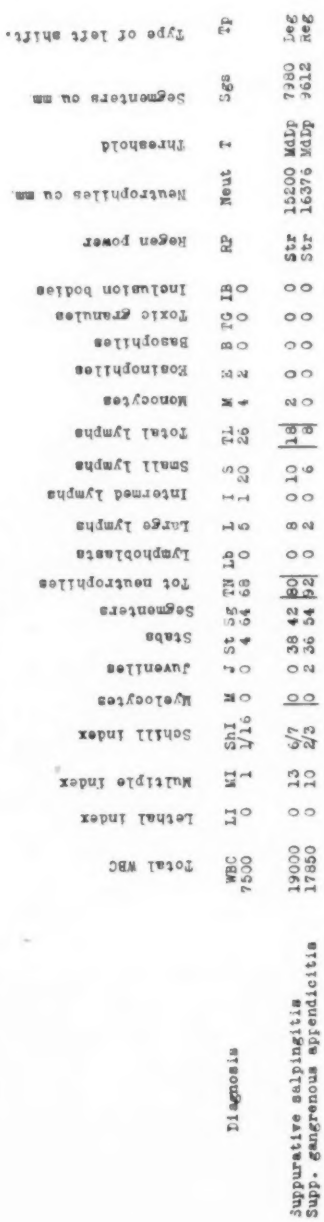

Fig. 13.—Hemograms showing differential diagnosis of processes that are similar clinically and of the same hemogramic types.

(A), however, shows but slight depression of the bone marrow threshold. The slight left shift is degenerative, the Schilling index a fraction; the multiple index only a few times greater than normal and the large army of neutrophiles consists almost entirely of efficient phagocytes or segmenters.

(B), on the contrary, shows many indications of toxic depression of the threshold and lowered resistance, consisting of a marked left shift, Schilling index greater than one and a multiple index many times greater than normal. The segmenters number less than half the total neutrophiles, so that while the cellular defensive force is large, it is of very poor quality.

The first hemogram is Type 5-B; the second is Type 2. Both were taken at the onset of disease before diagnostic clinical signs had developed. Depression of the threshold is not a dependable means of distinguishing between these two infections. Although the character of the shift differs in the two cases cited, it cannot be used as a differential point because the quality and quantity of the shift is often the same in the two diseases. The different action of the toxins on the reproductive power of the bone marrow is shown in the two hemograms. In influenza severe depression or lack of stimulation of the regenerative power results in a severe neutropenia with 44 per cent neutrophiles, or 2,420 per c.mm. In pneumonia strong stimulation of the regenerative power is shown by the 92 per cent, or 21,452 neutrophiles per c.mm., a marked neutrophilia. Thus a wide left shift, leukopenia, neutropenia, and lymphocytosis suggest influenza, while leukocytosis, neutrophilia, and lymphopenia, with a wide left shift, indicates pneumonia.

These hemograms are both Type 2, and are similar in practically all respects, except the degree of neutrophilia and lymphopenia. The hemogramic diagnosis in this case is difficult and often impossible. However, the total white count with 80 per cent neutrophiles, and 20 per cent lymphocytes and only a moderate degenerative left shift, is more characteristic of acute salpingitis than of appendicitis. If the first count had been due to acute suppurative appendicitis, the toxic depression of the threshold accompanying a total white count of 19,000 would have been greater. Thus, there would have been a wider shift, at least of the regenerative type, with a neutrophilia of probably 90 per cent and a corresponding lymphopenia. The second hemogram illustrates the type of shift that accompanies a high leukocytosis in acute appendicitis and shows the difference between the two.

COMMENT

We have endeavored to establish the following points:

1. The Schilling system of differentiating white blood cells should supercede all other forms of differential count. It reflects more consistently and accurately the degree of intoxication or infection of the patient.

2. Our modification of the Schilling hemogram has made possible an easier and more accurate analysis of the hemogram;

a. By adding a shiftograph to trace at a glance the extent of the quantitative shift indicated by the Schilling index.

b. By adding a multiple index to show the number of times a given shift is greater than normal.

c. By adding a lethal index to show the quality of the shift in a ratio of myelocytes or juveniles to segmenters, and to aid in prognosis. A fatal outcome almost always results when the lethal index approaches one and the multiple index 100.

d. By using the symbol "RP," to record the degree of stimulation, depression, or paralysis of the bone marrow, reflected by the number of neutrophiles per cubic millimeter.

e. By listing the neutrophiles per cubic millimeter to show the numerical strength of the neutrophilic army of defense.

f. By using the symbol "T" to record the condition of the bone marrow threshold, estimated by the quality of the shift. The degree of depression of the threshold when compared with the ratio of segmenters to neutrophiles per cubic millimeter, indicates the quality and quantity of the cellular army of defense.

g. By listing the segmenters per c.mm. for comparison, with average normals, and with neutrophiles per c.mm.

h. By routinely Schillingizing the lymphocytes a vast field of new information is opened up. Diseases of a chronic character, having a tendency to lymphocytosis, in the active phase show a lymphatic left shift with a predominance of large lymphocytes, and in the latent or healing phases a right shift to small lymphocytes.

In acute infections the first sign of the immunohealing phase of convalescence is an increase in lymphocytes and a lymphatic left shift with a predominance of large lymphocytes. As convalescence progresses the lymphocytes shift to the right with small lymphocytes predominating.

In tuberculosis the variation in total lymphocytes, the lymphatic shift in conjunction with the neutrophilic shift, and the rise and fall of monocytes and eosinophiles, definitely assist in classifying the various phases of the disease and in detecting complications.

i. By showing that the Schilling index alone should be used with caution as a diagnostic and prognostic aid. It represents the nonfilament-filament ratio, and is valuable only when considered with the quality of the shift.

The total number of shift cells alone may be used in differentially diagnosing acute appendicitis. In general with a neutrophilia present, and a Schilling index of one or over, shift cells 15 to 35 indicate acute catarrhal appendicitis; 35 to 50 suppurative appendicitis; 50 to 60 abscess in appendix; and over 60 ruptured appendix with general peritonitis.

j. By listing Doehle's inclusion bodies and Freyfeld's toxic granules in percentage of neutrophiles of all types containing them. These are found in any severe acute infection or intoxication and assist in determining the presence of septicemia.

3. Disease entities and different types of the same disease may be classified according to the type of hemograms which they show, thus facilitating diagnosis and prognosis.

4. Need for the shiftogram, a chart of sequential hemograms joined by the shiftograph, in following the daily progress of a disease. Definite changes in the course of an infection are usually indicated by a turn of the shiftograph often before there is any clinical change. During the course of a chronic infection an increase in neutrophiles with neutrophilic and lymphatic left shift indicates an acute exacerbation. A change in the severity of the picture during an acute infection may indicate a complication. Similarly a sudden turn of

the shiftograph and qualitative shift to the left, during a convalescent picture, may indicate a relapse. Remissions and attacks, in diseases characterized by them, may be similarly predicted by the change in the hemogram.

Lobar pneumonia seems to be an outstanding exception to the use of the shiftograph in predicting the turning point in the course of an infection. At the time of the clinical crisis the hemogram may show its greatest severity, the right shift not appearing until twenty-four to forty-eight hours later.

A shiftogram showing a sustained leukocytosis of from 10,000 to 20,000 with a neutrophilia of 8,000 to 18,000 per c.mm., a degenerative or regenerative left shift and Schilling index over one, is usually indicative of confined pus, e.g., abscess, empyema, etc.

A shiftogram has a place in the control of treatment by specific and nonspecific immunotransfusions, foreign protein therapy, diathermy, vaccines, antitoxins, etc. Many of these methods, which produce a febrile reaction clinically, also cause fairly characteristic changes in the blood picture and all of them may be more accurately controlled by the shiftogram.

A consideration of the clinical facts is important before an accurate hemogramic diagnosis and prognosis may be made. The clinical findings are important for prognosis in general because the hemogram has a different interpretation depending on the location of the infection. The same severity of blood picture that might indicate a fatal prognosis in intracranial infections, grave in pulmonary, serious but hopeful in abdominal, would cause but slight concern in pelvic conditions. Thus a slight shift in intracranial infections becomes of relatively great importance. A better prognosis may be given if it is known that adequate medical or surgical treatment is possible to control the primary focus of infection.

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A NOTE ON THE USE OF THE HAGEDORN-JENSEN BLOOD SUGAR TECHNIC IN CASES OF PHLORHIZINIZATION*

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DESPITE the generally accepted fact that the injection of phlorhizin causes a fall in blood sugar, examination of the literature reveals that comparatively high values have been reported, by Coolen,[1] Pavy,[2] Biedl and Koliseh,[3] Massaut,[4] and Houssay and Biasotti.[5] Yamada[6] seems to have been the first definitely to associate high values with reducing action of phlorhizin itself rather than with any glycemic action of the drug. He found an apparent rise of blood sugar as determined by the Hagedorn-Jensen ferricyanide method, but no change for several hours with Bang's copper reduction technic. Lundsgaard[7] precipitated the blood proteins with mercuric sulphide, and found that phlorhizin was removed by this procedure.

METHODS

Total reducing substances were determined by the Hagedorn-Jensen technic,[8] following a Somogyi zinc precipitation.[9] Nonfermentable reducing substances were determined on the zinc filtrate after fermentation with yeast according to Peters and Van Slyke.[10]

EXPERIMENTAL

The error caused in the Hagedorn-Jensen method is twofold: the phlorhizin reduces ferricyanide and raises the titration value by combining with iodine. The present work is an attempt to study and rectify both of these errors.

To determine the iodine absorption, a 0.1 per cent water suspension of phlorhizin (Merck) was used in place of blood. After the Somogyi precipitation, 1 c.c. samples were placed with 1 c.c. portions of an iodine solution for five, ten,

*From the Laboratory of Physiology, Yale University. The School of Medicine.
Received for publication, January 24, 1934.

and fifteen minutes, both at 25° C. and at 18° C. The titration values with 0.005 N $Na_2S_2O_3$, as used in the Hagedorn-Jensen technic, are given in Table I.

TABLE I

IODINE ABSORPTION PER C.C. OF 0.1 PER CENT PHLORHIZIN FILTRATE

	IODINE ABSORBED AT 25° C. (AS C.C. 0.005 N $Na_2S_2O_3$)	IODINE ABSORBED AT 18° C. (AS C.C. 0.005 N $Na_2S_2O_3$)
After 5 minutes	0.10	0.02
After 10 minutes	0.22	0.02
After 15 minutes	0.24	0.04

Examination of Table I shows that good cooling and speedy titration will minimize the error due to absorption of iodine by phlorhizin.

Using the information thus obtained, 1 c.c. samples of the filtrate were then subjected to the entire technic for the detection of reducing substances, both before and after fermentation. The values obtained are presented in Table II.

TABLE II

REDUCING POWER PER C.C. OF 0.1 PER CENT PHLORHIZIN SUSPENSION

	REDUCING POWER (AS MG. GLUCOSE)
Total	0.39
After 10 minutes' fermentation	0.36
After 15 minutes' fermentation	0.37
After 20 minutes' fermentation	0.36

Phlorhizin, therefore, has a constant reducing power even after as much as twenty minutes' fermentation. The disastrous effects of over fermentation are shown in Table III, which contains reducing values of blood filtrates after one-half hour with yeast. The data show that fermentation of the phlorhizin must have taken place.

TABLE III

REDUCING SUBSTANCES IN BLOOD WITH PHLORHIZIN, AFTER THIRTY MINUTES' FERMENTATION (AS MG. GLUCOSE PER C.C.)

PHLORHIZ. MG./C.C.	TOTAL REDUCING CONTROL	TOTAL REDUCING EXPER.	FERMEN. REDUCING CONTROL	FERMEN. REDUCING EXPER.
1	0.77	1.12	0.73	0.85
2	0.77	1.46	0.73	0.92
3	0.77	1.81	0.73	1.00
4	0.77	2.19	0.73	1.12

Putting to use the facts shown by these experiments, namely, that phlorhizin is not fermented in fifteen minutes, and that at a lowered temperature it absorbs very little iodine, the effect of phlorhizin on whole blood was next determined. For this, oxalated slaughter-house blood, to which known amounts of phlorhizin had been added, was used, total and nonfermentable reducing substances being determined. Table IV contains the values obtained.

The dose of phlorhizin usually administered to animals is 1 gram per 10 kilos of body weight. If this entire amount passed at once into the blood stream, the maximum concentration there would be about 1.3 mg. per c.c. If the entire dose became distributed throughout all the tissues, the minimal concentration

TABLE IV

REDUCING SUBSTANCES IN BLOOD WITH VARYING AMOUNTS OF PHLORHIZIN (AS MG. GLUCOSE PER C.C.)

PHLORHIZIN MG./C.C. (1)	TOTAL REDUCING SUBSTANCES CONTROL (2)	TOTAL REDUCING EXPER. (3)	DIFFERENCE (3)-(2)	NON-FERMEN. REDUCING CONTROL	NON-FERMEN. REDUCING EXPER.	FERMEN. REDUCING CONTROL (4)	FERMEN. REDUCING EXPER. (5)	DIFFERENCE (5)-(4)
0.10	1.13	1.15	0.02	0.10	0.14	1.03	1.01	−0.02
0.10	1.24	1.24	0.00	0.11	0.11	1.13	1.13	0.00
0.13	1.13	1.15	0.02	0.10	0.12	1.03	1.03	0.00
0.13	1.24	1.29	0.05	0.11	0.17	1.13	1.12	−0.01
0.17	1.13	1.17	0.04	0.10	0.14	1.03	1.03	0.00
0.17	1.29	1.34	0.05	0.11	0.18	1.18	1.16	−0.02
0.20	1.13	1.19	0.06	0.10	0.14	1.03	1.05	+0.02
0.25	1.13	1.19	0.06	0.10	0.16	1.03	1.03	0.00
0.25	1.29	1.35	0.06	0.11	0.16	1.18	1.19	+0.01
0.33	1.24	1.35	0.11	0.11	0.14	1.13	1.11	−0.02
0.33	1.24	1.36	0.12	0.11	0.24	1.13	1.12	−0.01
0.50	1.08	1.27	0.19	0.14	0.30	0.94	0.97	−0.03
0.50	1.29	1.45	0.16	0.11	0.26	1.18	1.19	+0.01
1.00	1.08	1.41	0.33	0.14	0.48	0.94	0.93	−0.01
1.00	0.48	0.77	0.29	0.05	0.32	0.43	0.45	+0.02
1.00	0.48	0.79	0.31	0.05	0.36	0.43	0.43	0.00
1.50	1.29	1.72	0.43	0.11	0.51	1.18	1.21	+0.03
1.50	1.29	1.77	0.48	0.11	0.55	1.18	1.22	+0.04
2.0	1.75	2.36	0.61	0.14	0.67	1.61	1.69	+0.08
2.0	1.08	1.72	0.64	0.14	0.71	0.94	1.01	+0.07
4.0	1.75	2.96	1.21	0.14	1.21	1.61	1.75	+0.14
6.0	1.75	3.38	1.63	0.14	1.81	1.61	1.85	+0.24

of phlorhizin in the blood would be about 0.1 mg. per c.c. The amounts of phlorhizin added to the blood were calculated to cover the range one would expect to encounter after phlorhizinization.

It has been shown that the presence of phlorhizin in blood causes an apparent increase in sugar content, under the usual conditions for the Hagedorn-Jensen determination. This fact casts considerable doubt upon the accuracy of blood sugar values determined by the Hagedorn-Jensen method during phlorhizin injections.

SUMMARY

1. Phlorhizin introduces two errors into blood sugar determination with the Hagedorn-Jensen technic: first, it reduces ferricyanide, and, second, it combines with iodine.

2. Fermentation with yeast eliminates the first error, while rapid titration at 18° C. reduces the second.

3. Data are presented to show that the Hagedorn-Jensen method, with the modifications noted, may be used to determine fermentable reducing substances in blood after phlorhizinization.

The author wishes to acknowledge gratefully much valuable advice given by Dr. H. E. Himwich during the course of the work.

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A STABLE STARCH INDICATOR FOR THE IODOMETRIC ESTIMATION OF CHLORIDES IN BLOOD AND URINE*

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THE iodometric method for chlorides in blood is known to possess many advantages over the thiocyanate procedure.[1] It has been found applicable to urines also with high degree of accuracy.[2] It has, however, one objectionable feature, namely, the rapid deterioration of the starch indicator. The condition of the starch has been found to affect the accuracy of this method, since the end-point is sharp only when the starch is freshly made.[3] It is obvious, therefore, that a stable starch indicator would add to the reliability and convenience of this method.

A number of preservatives for starch indicators has been described, but, with the possible exception of salicylic acid,[1] they seem unsuitable for the special case of iodometric estimation of chlorides in biologic fluids.

In our experiments the urine preservative containing acetyl-salicylic acid and hexamethylenetetramine[4] was used as a starch stabilizer with very good results. It did not interfere with the iodometric method for chlorides in any particular. The preserved starch solutions were found to remain stable and efficient as indicators, without any special precautions being necessary. They did not develop any odor and were about as clear as a freshly prepared starch solution. A negligible amount of white precipitate formed in the course of time, but in no way interfered with the end-point. These solutions were kept at room temperature, since in the ice box their contents were found to precipitate out.

The preserved starch indicator was tested in a number of iodometric determinations of chlorides and checked against a freshly prepared starch solution. At the time of the test the preserved starch was from three to five months old, while the fresh starch was renewed every two days. The behavior of the two indicators was compared in standardization of the KI solution, in the analysis of NaCl controls and in the estimation of chlorides in blood and urine specimens. The results obtained show very close agreement and are given in Table I.

In these experiments some of the blood specimens contained NaF and monochlor-benzene as preservative.[5] All urine specimens were preserved with

*From the Laboratories of the Life Extension Institute.
Received for publication, January 29, 1934.

The starch used in these experiments was obtained from the Eimer and Amend Co.; the hexamethylenetetramine and acetyl-salicylic acid came from Merck & Co.

200 mg. each of acetyl-salicylic acid and hexamethylenetetramine per 100 c.c. of urine.[4] The last two substances were also added to a part of control NaCl solutions in the same concentration. The urines were all negative for albumin and the preliminary precipitation of the urine proteins was omitted.[2]

REAGENTS

In view of possible interaction of the preservative with the buffer salts (sodium citrate and sodium nitrite) on long standing, it was thought safer to prepare the indicator in the form of two solutions (A and B) as recommended by Short and Gellis.[6] To Solution A was added the above preservative. Solution B was left unchanged. The rest of the reagents were the same as used by the above authors and their procedure was followed for both blood and urine.

*Solution A (Preserved Starch).**—Bring 100 c.c. of water to boiling. Take off flame and immediately dissolve in it 2.5 gm. of soluble starch with constant stirring. Cool and dilute to 150 c.c. To the 150 c.c. of the cold solution add 510 mg. of acetyl-salicylic acid U.S.P. and 510 mg. of hexamethylenetetramine U.S.P. Stir the mixture thoroughly to insure complete solution. Let stand overnight. By that time some solid material that may be left undissolved will settle to the bottom. Centrifuge for ten minutes at high speed. Decant carefully the supernatant fluid. Keep at room temperature, tightly corked when not in use.

Solution B (Buffer Salts).—Dissolve 466 gm. of crystalline sodium citrate and 20 gm. of sodium nitrite in about 800 c.c. of water. Dilute to 1,350 c.c. The two solutions are mixed just before use, in proportion of 1 c.c. of A to 9 c.c. of B.

Standard Silver Nitrate Solution.—Dissolve 5.812 gm. of $AgNO_3$ in about 400 c.c. of water, using an 800 c.c. beaker. Add exactly 250 c.c. of HNO_3 (sp. gr. 1.42). Cool to room temperature. Transfer to a liter flask and dilute to mark with water.

Standard Potassium Iodide Solution M/73.1.—Dissolve 2.4 gm. of KI in a liter of water. Standardize by titrating against 5 c.c. of the $AgNO_3$ solution, to which have been added 5 c.c. of water and 6 c.c. of the starch indicator. Adjust so that the amount required will be 12.65 c.c. (12.50 c.c. to precipitate the silver nitrate and 0.15 c.c. to develop a definite end-point).

PROCEDURE

Into a 50 c.c. graduated tube introduce 20 c.c. of Folin-Wu blood filtrate and about 15 c.c. of water (or 1 c.c. of urine and about 35 c.c. of water). Add 10 c.c. of $AgNO_3$ solution. Dilute carefully to the mark with water. Mix several times by inversion. Centrifuge for ten minutes at high speed and decant the supernatant fluid through a filter. Titrate 20 c.c. of this filtrate (equivalent to 0.8 c.c. of blood, or to 0.4 c.c. of urine) with the KI solution, using 6 c.c. of the starch indicator. The KI solution is added slowly with constant stirring. Toward the end it is added drop by drop. A white porcelain dish instead of a glass vessel is used for titration. The yellow AgI precipitate serves as the correct background for the end-point, which is the *first* permanent blue.[1] In our

experiments enough of the final filtrate was obtained for duplicate determinations, one of which was made with the preserved starch, the other with the freshly prepared starch.

Calculation.—

In the case of blood the formula is:

(10.15 – c.c. of KI sol. used) × 100 = mg. NaCl per 100 c.c. of blood.

In the case of urine the factor is doubled:

(10.15 – c.c. of KI sol. used) × 200 = mg. NaCl per 100 c.c. of urine,

since the amount of urine taken is only half that of blood.

TABLE I*

COMPARISON OF PRESERVED AND FRESH STARCH INDICATORS

	Starch	
Standardization of KI	Preserved	Fresh
c.c. KI used in titration	12.24	12.25
	12.65	12.63
Value required = 12.65 c.c.	12.67	12.65
per 5 c.c. of st. $AgNO_3$ sol.	12.67	12.65

CONTROL NaCl SOLUTIONS MG. PER 100 C.C. OF SOLUTION		GIVEN	STARCH PRESERVED FOUND	STARCH FRESH FOUND	STARCH PRESERVED % ERROR	STARCH FRESH % ERROR
Theoretical error (varies with concentration): ±1% to ±3%	(a)	1,000	998	994	–0.2	–0.6
	(b)	1,000	1,002	998	+0.2	–0.2
	(a)	500	494	497	–1.2	–0.6
	(b)	500	496	494	–0.8	–1.2
	(a)	250	250	256	0	+2.5
	(b)	250	250	254	0	+1.6

(a) Pure NaCl solutions. (b) NaCl solutions containing acetyl-salicylic acid and hexamethylenetetramine.

BLOOD CHLORIDES MG. PER 100 C.C. OF BLOOD	STARCH PRESERVED FOUND	STARCH FRESH FOUND	STARCH PRESERVED % DEVIATION	STARCH FRESH % DEVIATION
The blood was preserved with NaF and Monochlor-benzene	504	501	0.6	
	473	477	0.8	
	539	535	0.8	
Theoretical deviation 1.2-1.5%	514	516	0.4	
	405	413	1.8	
	387	382	1.3	

URINE CHLORIDES MG. PER 100 C.C. OF URINE	STARCH PRESERVED FOUND	STARCH FRESH FOUND	STARCH PRESERVED % DEVIATION	STARCH FRESH % DEVIATION
The urine was preserved with acetyl-salicylic acid and hexamethylene-tetramine	644	652	1.3	
	910	910	0	
	546	546	0	
	260	266	2.5	
Theoretical deviation 1-3%	450	450	0	
	700	698	0.3	
	558	550	1.6	

*The preserved starch was 3-5 months old during the experiments. It was preserved with acetyl-salicylic acid and hexamethylenetetramine.

NOTE: The fresh starch was renewed every two days.

DISCUSSION

Inspection of Table I indicates that acetyl-salicylic acid in combination with hexamethylenetetramine forms a dependable preservative of starch solutions used as indicators. The theoretical error is given in the table. In the estimation of chlorides it varies with concentration of NaCl. It is equal to $\pm 0.6 - 1.5$ per cent when 2 c.c. of material is used and is nearly doubled when 1 c.c. is taken for analysis. In our experiments the controls were done in the same way as the urines, 1 c.c. having been used; accordingly, the error here is the same. As to bloods and urines, the deviation between results obtained with the two indicators amounts in our case to the difference between duplicate titrations. It can be seen that the agreement is within theoretical limits.

SUMMARY

A stable starch indicator was prepared with the use of acetyl-salicylic acid and hexamethylenetetramine as preservative. It was found to keep in the original state for several months. Comparative tests indicate that it can be safely used in iodometric methods for chlorides in blood and urine. The tests further show that this indicator works well when the specimens themselves contain the above mentioned preservatives.

I wish to thank Dr. James J. Short, Director of Laboratories of the Life Extension Institute, New York, N. Y., for having given his help and encouragement in this work.

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A SIMPLE METHOD FOR THE MANIFOLDING OF KYMOGRAPH TRACINGS*

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IT IS often found desirable to have copies of kymograph tracings for class distribution, and as a means of preserving the original, a method will be described whereby any number of copies may be made photographically without the necessity of using film or plates or camera.

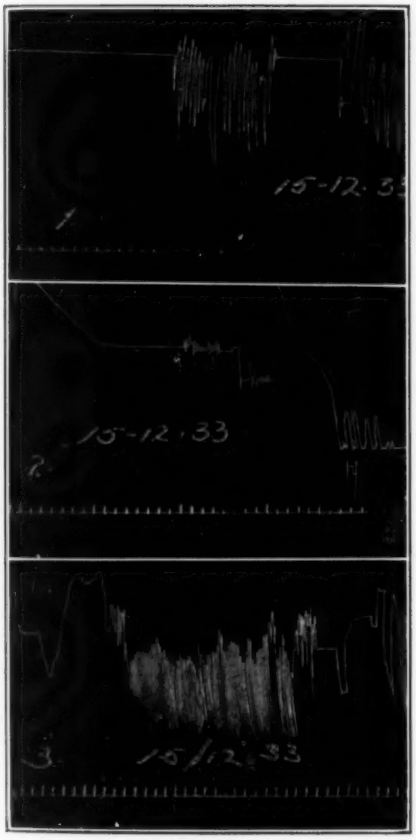

Fig. 1.

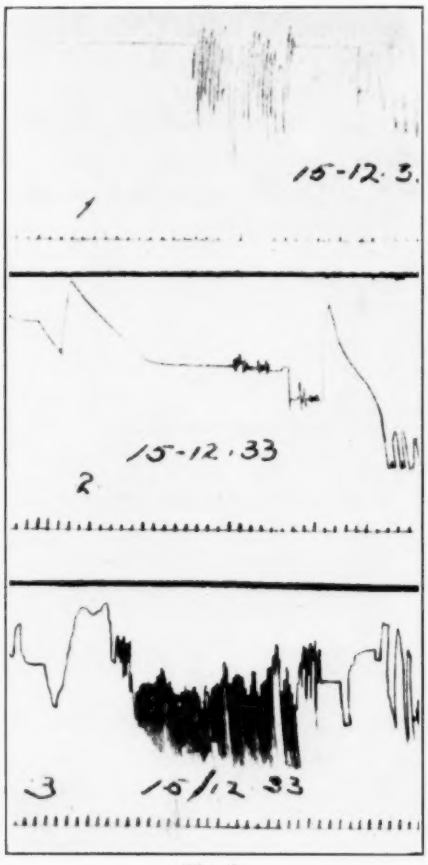

Fig. 2.

Fig. 1.—Drum smoked as described. Tracing 1, shellacked with a thin, fairly clear shellac. Tracing 2, shellacked with a heavier fairly clear shellac. Tracing 3, shellacked with shellac made according to the formula.

Fig. 2.—Grade F No. 5 AZO exposed for thirty seconds and developed for 1.5 minutes. Tracing 1 shows a thin shellac does not render the kymograph record paper suitable for good reproduction with average exposure and development.

The method depends upon the fact that when a tracing is made on a properly smoked drum paper, and is carefully fixed with a suitable shellac, the

*From the Department of Physiology, The Johns Hopkins Medical School.
Received for publication, January 3, 1934.

record may be used directly as a negative, and contact prints may be made therefrom.

An outline of the complete procedure follows:

1. *Smoking the Drum Paper.*—The paper should be smoked evenly and densely, the usual care being taken not to char the paper. A yellow Bunsen burner flame is very satisfactory.

2. *Shellacking the Tracing.*—It is essential for good copies that the proper grade of shellac be used. Reference to Tracing 1 in Fig. 1 will show the reason for this. The tracing should be passed through the shellac slowly and evenly, but only once. In our laboratories we have found the following shellac to give excellent results: Enough white granular shellac to occupy one-third of an eight-liter bottle is shaken with 250 c.c. of floor varnish and with 5,084 c.c. of 96 per cent grain alcohol and allowed to stand in a warm place for a month with occasional shaking. The clear liquid is then decanted and 5 c.c. of Venetian turpentine and 100 gm. of gum mastic are added, the mixture is shaken, allowed to stand, and the clear portion is siphoned off for use.

3. *Printing the Duplicates.*—See Table I for choice of paper. Any type of printer may be adapted. A very simple one may be made by placing a light

TABLE I

USE	NAME OF PAPER	EXPOSE*	DEVELOP
For further photographic reproduction, calibration, etc.	Apex S No. 55[1]	About 2 minutes	About 2 minutes
Glossy prints	Grade F. No. 5 AZO[2]	About 30 seconds	1.5 to 2 minutes
For notebook and classroom use where glossy surface is not necessary	Portrait proofing paper[2]	Less than 1 second	10 to 15 seconds
For enlarging from tracings	Apex S No. 55[1]	About 4 minutes	About 3 minutes
	PMC Bromide No. 10	About 2 minutes†	About 2 minutes
	Glossy—extra contrast[2]		

*Exposure times stated are for average tracing such as Tracing 3 in Fig. 1, with a 60 watt lamp (frosted) about three inches from tracing and paper.

†Will vary with type of projector used. Two to three minutes is average with 500 watt lamp.

[1]Defender Film, Du Pont Film Manufacturing Co., New York City.

[2]Eastman Kodak Co., Rochester, New York.

inside of a box with a plate glass top. The tracing is placed in the printer with the smoked side toward the light, and the sensitized paper is placed on top of the tracing, with the emulsion side also toward the light (see Table I for exposure and development time).

4. *Development.*—The paper is developed according to the type chosen. For the types of paper listed I have found the Elon-Quinol developer, put up in tubes by the Eastman Kodak Company, to be very satisfactory.

5. *Fixing.*—All papers should be fixed for at least twenty minutes in the usual acid hypo bath.

6. *Washing of Prints.*—All prints should be washed for at least three-quarters of an hour in running water or in several changes of clean water.

The procedure as outlined is for the quick and inexpensive duplicating of records. Should facsimile duplicates be desired, a negative must first be

made on any good orthochromatic film, or a direct positive paper may be used. This, of course, adds to the time and expense necessary for turning out the duplicates.

SUMMARY

It is often desirable to have duplicates of kymograph tracings for calibration. A method is described whereby photographic duplicates may be easily obtained without the use of film, plate, or rephotographing camera.

ON THE TECHNIC OF MEULENGRACHT'S ICTERIC INDEX DETERMINATION*

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WHEN serum is diluted with 0.9 per cent sodium chloride solution, turbidity occurs rather frequently and complicates accurate comparison of the serum and standard bichromate solution.

Although determination of the icteric index is a very common procedure, little mention has been made of this difficulty. Reinhold[1] claims that clearer solutions are obtained if a phosphate buffer solution of pH 7 is used as a diluent. The writer finds this a valuable improvement. Perhaps an even simpler method of overcoming the difficulty consists of diluting the serum with distilled water and adding one drop of a 10 per cent solution of sodium hydroxide.

Adding a similar amount of sodium hydroxide to acetone solutions of bilirubin markedly alters their color, but no similar change occurs in the case of serum, probably because buffers are present and other conditions are different. In a series of determinations the results were identical whether sodium chloride solution, phosphate buffer solution of pH 7, or distilled water and sodium hydroxide solution in the above amount was used as diluent. The slight modification which is suggested in the test has been found useful in numerous routine determinations of the icteric index and is, therefore, communicated to other laboratories.

*From the Department of Laboratories, Henry Ford Hospital.
Received for publication, February 12, 1934.
[1]Cited by Hawk and Bergeim: Practical Physiological Chemistry, ed. 10, p. 446.

NOTES ON THE APPLICATION OF BISMUTH SULPHITE MEDIUM TO THE ISOLATION OF *B. TYPHOSUS* FROM FECES*

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AN INVESTIGATION of Wilson and Blair's bismuth sulphite medium at the laboratories of the Georgia State Board of Health indicates that this medium promises to be a most valuable adjunct in the work of isolating *B. typhosus* from feces for purposes of diagnosis and of detection of carriers. We have been able to find little or no reference to this medium by American investigators. We are, therefore, citing our experience with it for the benefit of those who may be interested in giving it further trial.

Wilson found in 1923 and 1926 that "*B. typhosus* in the presence of a fermentable carbohydrate is able to reduce a sulphite to a sulphide and that a combination of bismuth and sodium sulphite affords an enrichment and selective medium for *B. typhosus* and at the same time partially or completely suppresses the growth of *B. coli.*"

Wilson and Blair in 1927[1] (see also 1931)[2, 3] incorporated this principle in a solid agar medium. While this medium has since undergone certain modifications, we have employed so far the original "Old Standard" formula as follows:

1. *Stock Agar Base.*—

Shredded agar (A.H.T.40)	30 grams
Peptone (Bacto or Fairchild)	10 grams
Meat extract (Liebig or Armour)	5 grams
NaCl	5 grams
Water	1,000 c.c.

Prepare in the usual way.

Note: No adjustment of the reaction is necessary.

2. *Bismuth Liquor.*—A glass stoppered bottle is almost filled with 500 c.c. water and the fluid level is marked on the side of the bottle. After pouring out the water, 60 gm. of bismuth citrate is introduced, followed with 50 c.c. of distilled water. By means of a glass rod the citrate is made into a thin paste with the water; 20 c.c. strong ammonia water is then added and stirring is continued. The resulting chemical reaction produces some heat. The glass stopper is replaced and the bottle shaken until the bismuth is almost completely dissolved.

Note: The formula calls for ammonia water of specific gravity 0.880. We found that 20 c.c. of the ammonia water we used was insufficient, but by adding more, a few drops at a time, a satisfactory solution was finally obtained. This was not water clear but had a smoky appearance.

After dissolving the bismuth, the bottle is filled up to the 500 c.c. mark. The final appearance of the bismuth liquor is slightly opalescent and upon long standing a fine precipitate settles out. This should be shaken up before using.

*From the Laboratories of the Georgia State Board of Health.
Received for publication, February 9, 1934.

3. *Preparation of Medium.*—

(a) To each 100 c.c. of melted stock agar add:
5 c.c. of 20% solution of dextrose
10 c.c. of 20% solution of sodium sulphite (dried)
5 c.c. of the bismuth liquor.

(b) Immerse the container in boiling water for two minutes, remove and add:
1 gram of di-sodium phosphate (anhydrous) which has previously been dissolved in 10 c.c. boiling water.
1 c.c. of an 8 per cent solution of ferrous sulphate anhydrous.
0.5 c.c. of a 1 per cent aqueous solution of brilliant green.

(c) After thoroughly mixing, pour at once into sterile Petri dishes about 10 to 15 c.c. to each dish. Allow to harden and cool with the cover ajar. Then replace covers and store in the ice box or use at once as required.

Note: The following chemical reagents used by us seemed satisfactory:
Ammonium hydroxide—C.P. Sp. Grav. 0.90.
Sodium sulphite, dried Na_2SO_3 Baker.
Di-sodium phosphate anhydrous Na_2HPO_4 Baker.
Bismuth citrate U.S.P. Merck.
Brilliant green, Gruebler.
Ferrous sulphate, anhydrous, Baker.

The finished product after cooling in the plates is of a pale greenish gray color, semi-opaque, and of a flaky texture, although the surface is smooth.

Inoculation of Plates.—Since most strains of *B. coli* are almost completely suppressed, the plates may be inoculated much more heavily than when using endo or eosin methylene blue plates. Three or four loops may be spread over each plate, taking care not to tear up the surface.

While growth of *B. typhosus* and certain other organisms may be visible after twenty-four hours' incubation, the colonies are more sharply differentiated after forty-eight hours. In the absence of *B. typhosus*, plates planted with feces are usually completely blank after forty-eight hours and can be discarded at a glance. Occasionally growth of organisms other than *B. typhosus* (see below) will occur, but it is seldom that these need be confused with that of the latter.

The typhoid colony when well isolated appears after forty-eight hours as rather small dry flat and very black with a metallic luster. It is surrounded by a blackish smoky halo of from one to three mm. radius. In areas of the plate where the colonies are quite close together the description as given above does not apply so constantly and the halo effect may not appear.

In Table I the growth and description of colonies of the important members of the enteric group is given. In the first column are the observations made by Wilson and Blair.[2] In the second column are listed the observations made by us in our own experience. Both observations were made after forty-eight hours' incubation of the plates.

Of the organisms listed in Table I, the growth and colony formation of *B. typhosus* is by far the most characteristic. On the other hand, in culturing fecal specimens on bismuth sulphite medium not every colony that is metallic black flat and dry and surrounded with a dark halo proves to be *B. typhosus.* We have occasionally encountered a variety of organisms which are capable

TABLE I

BISMUTH SULPHITE MEDIUM

ORGANISM	DESCRIPTION OF GROWTH AND COLONIES AS NOTED BY WILSON AND BLAIR[2]	DESCRIPTION OF GROWTH AND COLONIES AS NOTED BY THE AUTHORS
B. coli communis	No growth	No growth
B. coli communior	No growth	Not tested
B. Aerogenes	A few sticky colonies	Sticky moist elevated grayish colonies with light centers—no halo
B. morgan No. 1	Fine greenish colonies	Small pale green colonies
B. Alcaligenes	No growth	No growth
B. dysenteriae Flexner Shiga, Hiss Y	No growth	No growth
B. paratyphosus A	Clear dry colonies	Medium-sized light green colonies with darker centers
B. paratyphosus B	Large blackish colonies	Moist grayish brown confluent colonies
B. typhosus	Black metallic colonies with darkening of surrounding media	Black flat dry colonies with metallic dark halo

of producing colonies very closely resembling those produced by *B. typhosus*. As yet no attempt has been made by us to identify these except to satisfy ourselves that they were not *B. typhosus*. Wilson and Blair[3] found that at times *B. paratyphosus B* produces colonies similar to *B. typhosus*, as do also certain sulphite reducing strains of *B. coli*. The latter, however, are usually inhibited in growth by the addition of brilliant green. We propose to investigate these later. Such "false alarm" colonies are only occasionally encountered and can easily be ruled out. However, presumptive tests such as direct slide agglutination should not be relied upon as confirmation of *B. typhosus*.

All suspicious colonies are picked and transferred to Russell's double sugar tubes and to glucose, mannose, maltose, saccharose and lactose tubes. It is often advisable to use every differentiating device available before making a definitely positive identification of *B. typhosus*.

In Table II is shown a comparison of endo and bismuth sulphite media when plated with feces from known typhoid carriers.

TABLE II

KNOWN *B. typhosus* CARRIERS	ENDO PLATES	BISMUTH SULPHITE PLATES
Mrs. S.	Pure culture typical typhoid about 300 colonies	Pure culture—innumerable typical typhoid colonies
J. F.	6 typical typhoid colonies; numerous colonies *B. coli*	75 typical typhoid colonies; no *B. coli*
E. L.	About 100 typical typhoid; numerous colonies *B. coli*	Pure culture innumerable typical typhoid colonies
T. G.	6 typical typhoid colonies; numerous *B. coli*	Pure culture, about 100 typical typhoid colonies

In all four cases the growth of *B. coli* was entirely inhibited on the bismuth sulphite plates, but in three cases the growth of *B. coli* on the endo plates greatly interfered with the detection of typhoid colonies. The endo plates were inoculated with only a fraction of a loop of specimen, while the bismuth

sulphite plates were more heavily inoculated with four full loops of specimen.

In Table III the comparative results with endo and bismuth sulphite plates obtained from a series of selected routine fecal specimens is shown.

TABLE III

FECAL SPECIMEN NO.	ENDO PLATES	WILSON AND BLAIR'S BISMUTH SULPHITE PLATES
30	Numerous *B. coli* colonies, no typhoid	Many moist green colonies, no typhoid
31	Numerous *B. coli* colonies, no typhoid	No growth
1	Numerous *B. coli* colonies, no typhoid	Numerous small black colonies, no typhoid
2	Numerous typical typhoid colonies—and a few *B. coli* colonies	Innumerable typical typhoid colonies, no *B. coli*
3	Innumerable colonies of *B. coli* and related organisms—no typical typhoid colonies observed	30 typical typhoid colonies, numerous small light brown colonies, no *B. coli*
4	Numerous *B. coli* colonies	Many colonies suggestive of *B. aerogenes*, no typhoid
5	65 *B. coli*	Two small black colonies without halos, not typhoid
6	Numerous *B. coli*	Many black colonies without halos, not typhoid
101	100 *B. coli*	150 small black colonies without halos, not typhoid
22	250 *B. coli*	100 small black colonies without halos, not typhoid
8	50 typical typhoid and 30 *B. coli* colonies	200 typical typhoid colonies, pure culture
9	15 typical typhoid, 100 *B. coli*	About 300 typical typhoid, pure culture
10	75 typical typhoid, 100 *B. coli* colonies	200 typical typhoid colonies, pure culture
13	3 typical typhoid, numerous *B. coli*	Numerous typical typhoid colonies, pure culture
11	Numerous *B. coli*, no typhoid colonies seen	2 typical typhoid, 4 unidentified colonies
111	Numerous *B. coli*	About 300 small green colonies later found to be *B. morgan*
44	Numerous *B. coli*	No growth
222	Numerous *B. coli*	No growth
1111	50 *B. coli*, 6 *B. alcaligenes*	5 black colonies not typhoid
7	Numerous *B. coli*, no typhoid seen	Numerous typical typhoid colonies pure culture
88	Numerous large clear colonies resembling *B. alcaligenes*. Also numerous *B. coli*	100 typical typhoid, pure culture
99	Numerous *B. coli*, several clear colonies suggestive of *B. alcaligenes*	1 black colony with small halo but not typhoid. 1 large black colony without halo, not typhoid. 3 round black moist colonies, not typhoid

Of the 22 specimens listed in Table III, nine proved to contain *B. typhosus* with the bismuth sulphite medium of which only 6 were positive on the endo plates. In no instance was *B. typhosus* found on endo plates and not on bismuth sulphite. Some of these specimens were from known chronic carriers, others from convalescents and some from active cases. The finding of typhoid bacilli in Specimens 11, 3, 7, and 88 would very probably have been missed if endo medium only had been used.

As previously stated, not all typhoid-like colonies appearing after forty-eight hours on bismuth sulphite plates prove to be *B. typhosus*. Therefore the usual confirmation tests should be applied.

ADVANTAGES AND DISADVANTAGES OF BISMUTH SULPHITE MEDIUM

Advantages.—

1. Since *B. coli* is almost completely inhibited, colonies of *B. typhosus* have room to develop. The interference of *B. coli* growth on endo is often a serious handicap in the finding of typhoid colonies, especially if the latter are not numerous.

2. Bismuth sulphite plates may be heavily inoculated without fear of overgrowth. Obviously this enhances the prospect of finding *B. typhosus*. In examining large groups of food handlers, this is particularly advantageous, in that only one bismuth sulphite plate need be inoculated. In using endo plates it is necessary to inoculate several at once or one or two daily for three or more successive days, a procedure which requires considerable time and materials.

3. *B. morgan* No. 1 grows well on bismuth sulphite plates, producing rather characteristic green colonies. Our experience with this organism suggests that its pathogenic significance has not heretofore been appreciated. Morgans' bacillus also grows well on endo, producing clear colonies not unlike typhoid. However, it is easily overlooked on endo plates, due to overgrowth of *B. coli* and to the small amount of specimen inoculated.

Disadvantages.—

1. Bismuth sulphite plates require forty-eight hours' incubation before satisfactory observations can be made. Endo plates, on the other hand, may be read after twenty-four hours' incubation.

2. Bismuth sulphite plates, in our experience, may deteriorate after three or four days even at ice box temperature. *B. typhosus* will grow to some extent on old plates, but the colonies are not always characteristic. Thus it is not feasible to prepare, as in the case of endo, a supply of poured plates larger than the anticipated needs for three or four days.

3. Bismuth sulphite medium is not so satisfactory for the culturing of the paratyphoids and dysentery bacilli, with the exception of Morgan's bacillus which probably should not be classified with the *B. dysentery* group.

4. Bismuth sulphite medium is more difficult to prepare than endo or similar media. This fact, together with its instability after three or four days, limits its use to such laboratories as have frequent occasion to examine relatively large groups of dairy workers and food handlers for the purpose of detecting typhoid carriers.

CONCLUSIONS

The bismuth sulphite medium as devised by Wilson and Blair is shown to be superior to endo medium as a means of laboratory detection of *B. typhosus.*

The preparation of the medium, its practical application and its advantages and disadvantages are discussed.

Bismuth sulphite medium is especially recommended for use in state and municipal laboratories where the detection of typhoid carriers among food handlers and dairy workers is an important feature.

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A SOLUTION PRESSURE DOME FOR THE MAMMALIAN LABORATORY*

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FOR several years in our mammalian laboratory we have used a portable unit containing the various salt solutions (anticoagulative and physiologic) under pressure. The system has been so satisfactory from both the standpoint of utility and ease of manipulation, that we feel it should be offered for adoption.

The availability of a sensitive, low displacement value instrument such as the Tycos sphygmomanometer, lead immediately to its utilization for measuring those pressures which ordinarily are measured with manometers and other devices having a high displacement value. Some uses are illustrated in Figs. 3, 4, 5, and 6. For example, if during a blood pressure experiment direct readings are desired, the Tycos gauge may be cut into the circuit for a short period by manipulating stopcock *x*, Fig. 4. The systolic and diastolic pressures may be read off while the record is being made, and each experimental factor studied, may be dealt with directly in a quantitative way. When the apparatus is used to measure urinary or salivary secretion pressure, as illustrated in Fig. 6, the clamp at *x* on the outlet *K* of the apparatus is removed and applied at *x* on the flow-cannula. The low displacement value of the system allows for quick readings after which the clamps are restored and the flow again determined. This latter convenience has been found very advantageous in student work.

The apparatus may be used in parallel with a recording tambour (see Fig. 3), in closed circuit systems. Balloons in the stomach may be inflated to any desired tension, and the activity of the stomach thereupon recorded by a standardized tambour. By this method the force of the gastric activity may be recorded, and observed directly upon the Tycos gauge.

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Received for publication, February 23, 1934.

In experiments upon intracranial pressure in dogs (see Fig. 5), the pressure dome is kept in continuous connection with the cranial cannula. The citrate pressure unit is manipulated, however, exactly as in the case of blood pressure (Fig. 4).

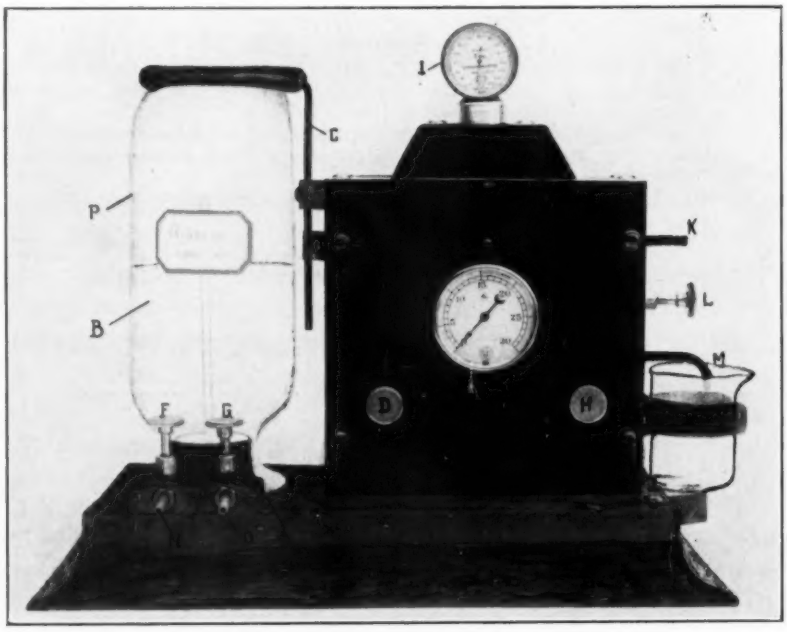

Fig. 1.—Photograph of solution pressure dome now in use. The function of each part can be understood by referring to the index letter of Fig. 2.

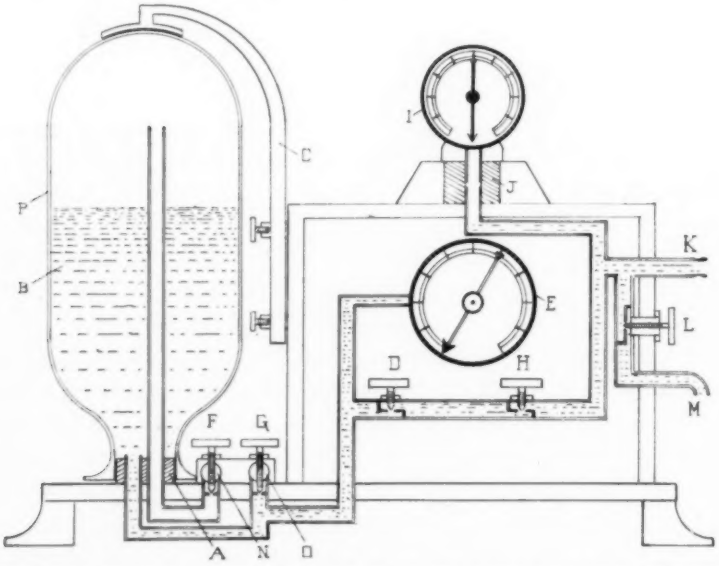

Fig. 2.—Drawing of solution pressure dome showing the parts used and their relation. For details see text.

The compressed air which is trapped in the dome is available through valve *F*, and occasionally, is found useful in clearing obstructions from cannulas

and syringe needles. These simple facilities available to the student have created a keen interest in the experiments, and have placed at his disposal means for making a number of direct observations of a fair quality of accuracy.

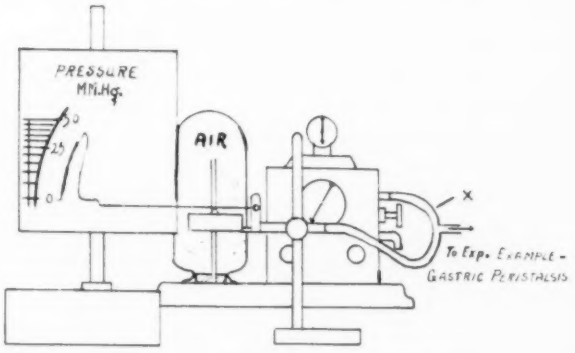

Fig. 3.—Standardizing large tambours.

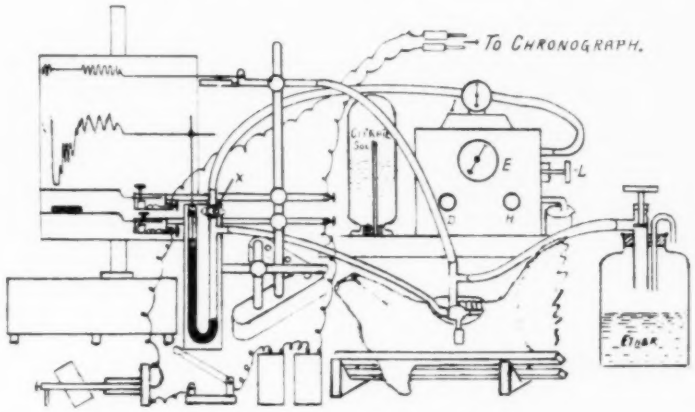

Fig. 4.—Blood pressure in dogs.

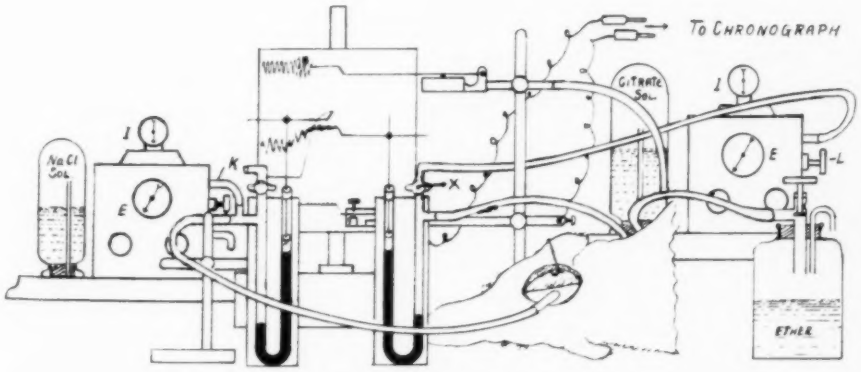

Fig. 5.—Intracranial pressure in dogs.

The Apparatus.—Figs. 1 and 2 illustrate the apparatus photographed, and in cross-section, respectively. The units used are easily assembled in any laboratory shop, and are available on the open market. They are: 1 two-liter

museum jar; 5 small needle valves (⅛ inch) or better, small valves made from compression couplings; 1 small steam gauge (3-inch face); 1 Tycos blood pressure gauge (taken from stock); 1 Tycos pump; the necessary pipe and couplings (obtainable from any plumbing supply house).

The entire unit costs but a few dollars and can be arranged neatly and mounted as desired. The apparatus as shown in Fig. 1 is mounted to take up very little table space, and to contain an ample charge of solution for average need. The jar (*P*) is charged with 1 liter of solution through intake (*O*) of valve (*G*). Above the solution a one-liter space serves as a compression chamber. The pressure in this chamber is built up to the desired point (about 6 pounds) through connection (*N*) of valve (*F*) by a hand pump taken from a blood pressure outfit. The pressure is read off directly upon gauge (*E*). This pneumatic cushion serves as the motive power to drive the solution (*B*) through the

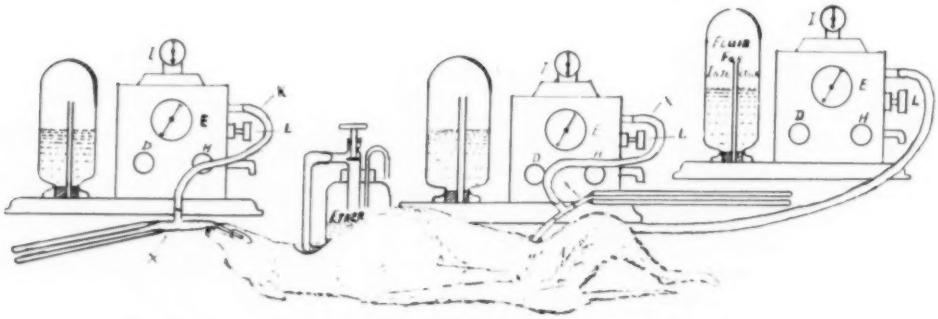

Fig. 6.—Salivary and urinary secretion pressures and fluid injections.

entire system. Two valves (*D*) and (*H*) in series provide convenient manipulation and control of the solution. (Valve *D* controls the rate of flow, while valve *H* serves primarily as a check, off or on.) By the use of this series system of valves one may set valve (*D*) at any given rate of flow, and return to it again and again. Outlet (*K*) is connected to the apparatus or system to be operated or tested and is always in parallel with pressure gauge (*I*). Valve (*L*) is a drain, and is used to release fluid from the system or to reduce the pressure built up through valves (*D*) and (*H*). (*A*) and (*J*) are rubber stoppers, which seal the museum jar (*P*) and the Tycos gauge mounting. By this system both the jar and the gauge may be removed instantly. The system is prepared for storage by emptying the jar and forcing through the system air under pressure. The systems come out of storage in good condition and are quickly placed into use.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SYPHILIS, Standard Treatment Procedure in Early: A Résumé of Modern Principles, Stokes, J. H., Cole, H. N., Moore, J. E., O'Leary, P. A., Wile, U. J., Parran, T. Jr., Vonderlehr, R. A., and Usilton, L. J. J. A. M. A. 102: 1267, 1934.

The résumé following is the product of a massive, world-wide investigation and represents the generally acceptable principles applicable to the management of this disease.

CONTROL OF INFECTIOUSNESS IN SYPHILIS

1. Infectiousness in syphilis is a function of three factors: (a) time; (b) arsphenamine; (c) individual predisposition to relapse.

2. Infectiousness is not a function of the serologic state of the patient. No serologic test has any value as a proof of infectiousness or noninfectiousness, early or late.

3. Syphilis, treated or untreated, is most infectious early, grows less with lapse of time, is rarely infectious (but may be so) after five years. Late syphilids and late prenatal (congenital) cases are not infectious.

4. Therefore, spar for time and delay in any issue involving infectiousness (i.e., marriage, intercourse).

5. Infectiousness is controlled and syphilis will be extinguished, if ever, as a health problem, by the treatment of the infectious person.

6. The public health responsibility of the physician is therefore with the early months and years of the disease.

7. Treatment to control infectiousness must be with the arsphenamines. No other drug will do.

8. The use of arsphenamines must not be delayed even to secure any minor individual immunizing advantage.

9. Treatment to control infectiousness must be continuous, not intermittent, and last at least eighteen months. Rest periods encourage relapse.

10. Acetarsone (Stovarsol), tryparsamide, and nonspecific (including fever) therapy must not be expected to control infectiousness.

11. Search for infectious lesions is ineffective, except as an adjunct. Hence inspection of prostitutes is useless.

12. Instruction and cooperation by the patient is ineffective and untrustworthy except as adjunct.

13. The amount of arsphenamine required is not less than twenty injections. The critical point is between 5 and 9.

14. Heavy metal is required as an adjunct.

15. The infectiousness of semen and vaginal secretions, even in the absence of lesions, in early and latent syphilis demands absolute mechanical protection in intercourse, treatment or no treatment. Continence, advised, is seldom practiced.

16. Inspection, instruction, control and protection are more essential in rest periods than under treatment.

17. There is a relapsing type of early syphilis regarding which no rules or predictions can be formulated.

18. In relapsing types, infectiousness may reappear immediately after, or even during (arsphenamine resistant), treatment with the arsphenamines.

19. Alcohol, dirt, bad hygiene, irritants including tobacco, sweat, friction (intercourse) predispose to infectious lesions.

20. The great promoter and sourse of relapse is the *short* arsphenamine course (one to four injections) unsupported by other treatment.

21. Treatment prophylaxis (after exposure) is unreliable. If given it must be followed through.

22. Adequate examination of patients would protect physicians, dentists, nurses and transfusion recipients from most risks of accidental infection.

23. The nonpregnant, nonsyphilitic woman should be protected mechanically and by treatment of the partner.

24. Conception should not take place except under treatment control.

25. The child of the pregnant syphilitic woman should not be destroyed, but protected in utero by treatment of the mother, before and after conception, and of the father, if syphilitic, before conception.

The term applied by Pusey to the superior outlook of treatment begun in the primary stage of syphilis before the blood serologic tests become positive (''seronegative primary'') has been justified abundantly by statistical analysis. The proportion of ''cures'' when treatment is begun in the seronegative primary stage (diagnosis by dark-field or other identification of *Spirocheta pallida*) is, as given, 71.4 per cent average and from 83 to 86 per cent best results. When, through failure of the patient to present himself or of his physician to diagnose primary syphilis until the blood test becomes positive, treatment is not begun until the so-called seropositive primary stage ''cure'' is attained in only 53.3 per cent by average and from 64 to 70 per cent by the best methods. This represents a clear loss of 18 per cent in outlook for ''cure'' by the delay. If the patient goes on to the development of a secondary eruption of course with a positive blood test, ''cure'' is attained in only 50 per cent by average and 61 to 82 per cent by best methods. This represents a possible loss of 21 per cent by average and 14 per cent by best methods over the outlook prevailing when treatment is begun in the seronegative primary stage.

It is accepted now as axiomatic that no patient with early syphilis should be treated by the sole use of an arsenical.

Just as no patient should be treated exclusively with an arsenical, so also no patient should be treated exclusively with a heavy metal, even with bismuth, for all that it possesses greater spirocheticidal qualities than mercury.

One of the most important contributions of recent years to the technic of treatment of early syphilis has been to the question of intermittence as distinguished from continuity of treatment.

It may now be said with positiveness that the old practice of administering treatment in early syphilis by fits and starts, conditioned on the Wassermann report of the blood, is pernicious; that even the introduction of a few weeks of complete rest from treatment into the management of the first eighteen months of the disease is likely to be profoundly injurious; that the patient who lapses or escapes treatment during this period is his own worst enemy; and that no rest intervals, and a regimen in which the patient is constantly receiving either an arsenical or a heavy metal during the first year of the disease or longer, if the indications require, is the best and safest modern practice, in the interest both of the patient and of the public health.

Continuous treatment, then, whether prolonged or brief, and practically regardless of the drugs used, is superior in its results to the intermittent or other schemes of treatment.

The failure of the blood Wassermann reaction to reverse is more a matter of how treatment is given than of how much treatment is administered. A little treatment continuously given is more than twice as effective as when intermittently applied and more than four times as effective as when irregularly given. Prolongation and intensification of treatment, however, using much arsphenamine and much heavy metal, but especially much arsphenamine in the first three months, promotes good results. Much arsphenamine and much heavy metal is four times as effective as little arsphenamine and little heavy metal in securing a negative Wassermann reaction within this period, when the drugs are continuously used.

A Scheme of Treatment for Early Syphilis

DAY OR WEEK	ARSPHENAMINE, GM.	INTERIM TREATMENT	BLOOD WASSERMANN REACTION	COMMENT
Day		--------	1	Arsphenamine dosage for first 3 injections at level of 0.1 gm. for each 25 pounds body weight; 0.4 gm. men; 0.3 gm. women; in average patient all lesions heal rapidly and blood Wassermann reaction becomes negative during first course; if arsphenamine cannot be used, substitute 8 to 10 doses 0.3 gm. silver arsphenamine, or 10 to 12 doses 0.6 gm. neoarsphenamine; this applies also to subsequent courses.
1	0.3-0.6			
5	0.3-0.6			
10	0.3-0.6			
Week				
3	0.4			
4	0.4			
5	0.4			
6	0.4			
7	0.4		1	If mercury is used note overlap of one week at end of first and start of second arsphenamine courses; at this point a few days without treatment may be dangerous; neurorecurrence.
8		Bismuth, 4 doses, 0.2 gm. and KI or Ung. Hg. and KI		
9				
10				
11				
12	0.4			Arsphenamine starts, bismuth stops; watch for provocative Wassermann reaction after first dose of arsphenamine.
13	0.4			
14	0.4			Try to prevent short lapses in treatment, especially at this early stage.
15	0.4			
16	0.4			
17	0.4			
18-23		Bismuth, 6 doses, or Ung. Hg. and KI	1	
24	0.4			Bismuth is better than mercury; use it if possible; examine cerebrospinal fluid at about this time if patient's cooperation can be secured.
25	0.4			
26	0.4			
27	0.4			
28	0.4			
29	0.4			
30-37		Bismuth, 8 doses, or Hg. and KI		
38	0.4		1	Note that bismuth or mercury courses are gradually getting longer—4, 6, 8 and now 10 weeks.
39	0.4			
40	0.4			
41	0.4			
42	0.4			
44-53		Bismuth, 10 doses, or Ung. Hg. and KI		
54	0.4		1	The average seropositive primary or early secondary patient should have at least 5 courses of arsphenamine.
55	0.4			
56	0.4			
57	0.4			
58	0.4			
59	0.4		1	
60-69		Bismuth, 10 doses, or Ung. Hg. and KI		It is safer to finish treatment with bismuth or mercury rather than with arsphenamine.
70-122		Probation. No treatment.	6-12	Blood Wassermann every month if possible, at least every other month.
123	Complete physical and neurologic examination, spinal puncture and, if possible, fluoroscopic examination of cardiovascular stripe. Thereafter, yearly physical examination blood Wassermann every six to twelve months; if the two spinal fluid examinations above are negative, this need not be repeated.			

The distressing frequency of the practice, for which physician or patient may be to blame, of giving four or five arsphenamine injections, testing the blood, finding the patient Wassermann negative in an early case, and virtually dismissing him with a few pills, inunctions or heavy metal injections should be fought with every resource that current knowledge can bring to bear.

It appears from the Cooperative Clinical Group investigation that the original arsphenamine when used alone is superior in rapidity of action on the blood Wassermann reaction; but the deficiencies of neoarsphenamine are to some extent compensated for by its use with a heavy metal and by its employment in a continuous rather than an intermittent system of treatment.

It appears that after two years of observation or treatment the largest number of patients obtaining satisfactory results falls in the category of those receiving from twenty to twenty-nine injections of an arsphenamine and a similar amount of heavy metal. Thus, thirty injections of the arsenical becomes a therapeutic objective, in place of the "forty or over" suggested by the results of earlier investigators.

REACTION PREVENTION PRINCIPLES

1. Inquire into the history of idiosyncrasy, allergic tendencies, skin irritability (especially eczema and seborrhea), focal and intercurrent infection, liver damage, and pregnancy before treatment is begun.

2. Question the patient before each treatment regarding (a) itching skin or rash; (b) purpura and melena; (c) gastrointestinal reaction; (d) condition of the mouth and teeth.

3. Examine at least the eyes (jaundice), face (dermatitis), mouth (salivation, bismuth pigment, purpura), flexures of the elbows (dermatitis), wrists and ankles (purpura) before each treatment. Take the temperature.

4. Make the first dose of any drug not more than half the full dose.

5. Pull back on syringe pistons: before intravenous injections to be sure of vein entry; before an intramuscular injection to be certain a deep vessel has not been entered.

6. Inject intramuscularly into the inner angle of the upper outer quadrant of the buttock and massage long and well after the injection.

7. Inject all solutions for intravenous use slowly through a small needle; not faster than 0.1 gm. per minute for neoarsphenamine.

8. Keep carbohydrate and alcohol low in the diet, and protein and fat high.

9. Permit only a light meal before and after an arsenical and prescribe a mild cathartic the morning after.

10. Make a urine examination biweekly.

11. Give calcium freely.

PHYSICIAN-PATIENT RELATIONSHIP AND RESPONSIBILITY

The physican should lay before the patient the facts under these heads:

1. That he has syphilis and present the evidence for the statement.

2. His outlook for "cure" with emphasis on the excellent reward of persistence in early cases.

3. The general facts of infectiousness as previously outlined.

4. The possibility of marriage, under medical control with personal cooperation, and the possibility of healthy offspring under prenatal direction and treatment.

5. The necessity for postponement or avoidance of marriage, intercourse, and pregnancy until the infection is under therapeutic control with the arsphenamines.

6. The relative inefficiency of the blood serologic tests as a measure of infectiousness, fitness for marriage or conception and "cure" (reiterate).

7. The dangers (relapse, neurorecurrence, serologic irreversibility and treatment-fastness, precocious tertiarism and malignant syphilis) of inadequate, short course, and irregular treatment.

8. The lulling into false security produced by the quick disappearance of symptoms under treatment.

9. The probable treatment requirements; including at least an eighteen months' estimate for early syphilis, whether seropositive or seronegative, primary or secondary.

10. Personal hygiene, control of treatment reactions, and symptoms of infectious recurrence.

11. The need for observation throughout life.

12. Special arrangements (examination of contacts and infection source; of family; communication of facts to others; treatment arrangements for travelers and nonresidents).

13. The probable costs and their settlement or adjustment.

TUBERCLE BACILLI, In the Gastric Contents of Tuberculous Children, Gourley, I. Am. Rev. Tubere. **29**: 461, 1934.

Fifty-nine cases of childhood tuberculosis have been studied by gastric lavage and guinea pig inoculation. All of these children reacted positively to the intracutaneous tuberculin test.

Fifty of the 59 patients had demonstrable lesions in the roentgenograms of the lungs. Nine had no demonstrable lesions. Their only evidence of tuberculosis was the positive tuberculin test.

Twenty-eight cases, 47.4 per cent, of the 59 were found to have tubercle bacilli in the gastric contents. Thirty-one cases, or 52.6 per cent, of the 59 cases studied, were negative for tubercle bacilli in the gastric contents. Of the 50 children with demonstrable lesions, 28, or 56 per cent, had positive gastric contents.

Fourteen children, or 50 per cent of the cases with positive gastric contents, were of school age. Should children with gastric contents containing tubercle bacilli be kept from close contact with healthy children?

Twelve children had calcium in the parenchyma of the lungs or regional lymph nodes. Five of these had positive gastric contents.

This study apparently indicates that all types of parenchymal lesions in childhood tuberculosis including the "C" group of Mc Phedran's classification are open cases. Further study is to be made before a definite conclusion can be reached.

TUBERCLE BACILLI, Demonstration of, in the Feces, Sputum, and Stomach Contents of Tuberculous Children, Mishulow, L., Kereszturi, C., and Hauptman, D. Am. Rev. Tubere. **29**: 471, 1934.

From the results obtained in this investigation, the following conclusions may be drawn:

1. Guinea pig inoculation should be made as a final confirmation when specimens are negative on smear examination.

2. Repeated examinations should be made in doubtful cases, inasmuch as positive and negative results are frequently obtained in the same case when the specimens are taken on different days.

3. The indications are that repeated examinations of feces by smear and guinea pig inoculation may prove as satisfactory as the examination of gastric lavage specimens.

4. As a diagnostic method, the examination of feces would be much more desirable than gastric lavage, as it could be easily repeated as often as necessary without any discomfort to the patient. Although gastric lavage examination has given very satisfactory results it is not always a desirable procedure, as it is hard on the patient when repeated examinations are made, and it is not feasible in some cases, especially when the patient is not hospitalized.

5. In the 60 cases in this series, the examination of feces, gastric contents, and sputa (when they were obtained) showed 10 per cent positive in children who were apparently well at the time of examination, 54.5 per cent positive in those that were slightly ill, and 63.1 per cent positive in the children who were very ill.

6. Of the children who had destructive parenchymatous pulmonary lesions, 83.3 per cent showed positive results and of those who had nondestructive parenchymatous lesions, 24

per cent were positive. Cases with only positive intracutaneous tests, hilum lymph node tuberculosis, pleurisy with effusion, and tuberculous peritonitis, were all negative.

TUBERCLE BACILLI, In the Sputum and Feces of Children Without Pulmonary Tuberculosis, Naïbant, J. P. Am. Rev. Tubere. **29**: 481, 1934.

Out of 19 children under fifteen years of age, 7 were found to have tubercle bacilli in the contents of their gastrointestinal tract. They had no demonstrable pulmonary tuberculosis.

BLOOD SEDIMENTATION RATE, Relationship Between Fibrinogen Content of Plasma and, Gilligan, D. R., and Ernstene, A. C. Am. J. M. Sc. **187**: 552, 1934.

One hundred and ninety simultaneous measurements of the fibrinogen content of the plasma and the "corrected sedimentation index" were made in normal individuals and in patients with various kinds and degrees of pathologic conditions.

A close correlation was observed between the plasma fibrinogen content and the corrected sedimentation index.

In certain cases with liver damage the corrected sedimentation index is increased disproportionately to the increase in plasma fibrogen content.

The results of the investigation indicate that, except in certain cases with liver damage, the plasma fibrinogen content plays the major rôle in controlling the corrected sedimentation index.

LEUKOCYTE COUNT, Effect of Hyperpyrexia Induced by Radiation Upon the, Bierman, W. Am. J. M. Sc. **187**: 545, 1934.

Hourly observations were made of the changes occurring in the leucocyte count of patients suffering from varied diseases in whom hyperpyrexia was induced by means of radio waves of 30-meter length.

An initial reduction, about 25 to 30 per cent, in the number of leucocytes regularly occurs, usually during the first or second hour of treatment. This is constantly followed by a leucocytosis whose maximum, amounting to about 80 per cent above the initial figure, occurs about the sixth to the ninth hour.

These variations are due mainly to changes in the total number of neutrophiles, of which the staff neutrophiles show the greatest increase. These changes, together with the appearance of other immature forms, indicate a stimulation of the bone marrow.

At a later stage the monocytes and lymphocytes also increase in number.

Repeated stimulation by heat is followed by a reduction in the leucocytic response.

ARTHRITIS, Rheumatoid, Autogenous Vaccines in, Short, C. L., Dienes, L., and Bauer, W. Am. J. M. Sc. **187**: 615, 1934.

Skin tests made with bacterial strains isolated from arthritic patients do not allow us to select specific strains for vaccine therapy.

Skin tests without using several subjects as controls are without any significance.

Variations in the skin reactions may be explained by differing irritability of the patient's skins, natural toxicity of the bacterial species, or possibly by a sensitization to certain bacterial groups.

The different methods which have been recommended for the selection of specific vaccine strains are without a solid theoretical or experimental foundation.

The above conclusions suggest that the therapeutic effect, if any, gained from autogenous vaccines is nonspecific.

The hypothesis that rheumatoid arthritis is a disease of allergic origin is not supported by conclusions drawn from the interpretation of uncontrolled skin reactions with autogenous vaccines.

LEUKOCYTE COUNT, Following Obstetric Analgesia Produced by Pentobarbital Sodium, Hardwick, R. S., and Randall, L. M. J. A. M. A. 102: 1558, 1934.

There has been nothing in the behavior of the mother or infant in any case in which pentobarbital sodium was administered to indicate any deleterious effect. Studies of the blood from this group of patients give no evidence of the production of a leucopenic condition with the amounts of pentobarbital sodium administered.

This series of studies of leucocyte and differential counts indicates that the greatest leucocytosis occurs at the fifth hour postpartum. The level of leucocytes falls steadily to the fourth day, when the number of leucocytes per cubic millimeter of the blood remains constant until the last examination on the tenth day.

TAKA-ARATA TEST, A Modified Mercuric Chlorid Reaction in Cirrhosis and Neoplasms of the Liver, Crane, M. P. Am. J. M. Sc. 187: 705, 1934.

Method.—Reagents: 0.9 per cent sodium chloride solution; 10 per cent sodium carbonate solution; 0.5 per cent mercuric chloride solution; 0.025 per cent aqueous fuchsin solution.

Into each of a row of 8 small glass tubes (those used for determining blood hemolysis are quite suitable) is placed 1 c.c. of 0.9 per cent sodium chlorid solution. To the first tube is added 1 c.c. of blood serum, ascitic or other fluid. From the mixed content of the first tube 1 c.c. is transferred to the second, the tube shaken, then 1 c.c. transferred from Tube 2 to Tube 3, and this procedure is continued throughout the series. In the eighth tube the 1 c.c. removed is discarded. The dilution of the serum or other fluid then ranges from 1 to 2 in the first tube to 1 to 256 in the last. To each tube is then added 0.25 c.c. of the sodium carbonate solution and 0.5 c.c. of the freshly prepared Takata reagent, which consists of a mixture of equal parts of the mercuric chlorid and aqueous fuchsin solutions. Readings are made immediately, and at one-half-hour and twenty-four-hour intervals. They depend entirely on the precipitate in the tubes. The precipitate at the end of twenty-four hours is almost invariably the most definite and constant, since precipitate which appears at earlier readings frequently diminishes or disappears on standing. Clouding and color changes are without importance; the same is true of the blue granular precipitate that sometimes occurs in the tubes of highest dilution, i.e., the seventh and eighth.

In view of the fact that we are concerned only with precipitation and that color changes play no part, the author has found it possible to omit the fuchsin. A further slight modification is the use of 6 tubes, rather than 8; for as previously mentioned, the precipitate in the last 2 tubes is without significance, and often occurs in distilled water alone.

The readings are made as minus (no precipitate, 1+, 2+, or 3+). Charting is of convenience. After moderate practice, charting is unnecessary and readings are negative, positive, or, for the sake of comparison in cases that are followed progressively, strongly positive.

The standard by which positive reactions are judged is the appearance of a definite precipitate in 2 of the first 3 tubes, and at the same time a precipitate in any number of the following tube, with the exceptions as previously noted. Positive reactions are usually quite characteristic, and negatives may have no precipitate in any tube, or a 1+ or a 2+ in any of the tubes from 4 to 7 alone.

Blood if freshly collected must be centrifuged; due care must be exercised to prevent hemolysis, as it is possible that this may cause false positives. The presence of bile is without influence. Blood or other fluids may be preserved almost indefinitely on ice or with toluol. Ascitic fluid that contains blood or pus must also be centrifuged.

The test is positive only in advanced cirrhosis, and is therefore of little value from the standpoint of early diagnosis.

Its chief worth lies in its differential diagnostic value to distinguish between cirrhosis and various liver neoplasms and other advanced liver disturbances.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Recent Advances in Sex and Reproductive Physiology*

PROFESSOR F. A. E. CREW, in the introduction to this book remarks that: "Man has turned from the adventurous conquest of his environment to the conquest of himself. Today is the day of biological invention, eagerly used for the control of the undesirable and the unwanted. Sex and reproduction are no longer hedged around by myth and taboo; they are no longer accepted as mysteries that defy understanding. They are matters inviting examination and explanation; they are regarded as expressions of physico-chemical forces, the nature of which is to be displayed. It is accepted that when knowledge is sufficient, control will be absolute, and, though knowledge is not yet sufficient, readers of this book must be persuaded to the view that this will not always be so."

The purpose of the volume is to present to the reader a survey of the present status of investigations concerned with the sex glands and the female sex hormones. As, of course, the greater part of the available information in this field is the result of animal research the work is naturally concerned with the studies made on animals and especially with those conducted on primates.

The volume deals essentially with the sexual and reproductive phenomena in the female in relation to the activity of the sex hormones.

It is an easy reading compilation and correlation of the work of many investigators which will serve the purpose of concentrating under one cover much of the literature reporting the investigations in this field.

A fairly extensive bibliography is appended which does not include, however, many American references of interest to students of this problem.

The book can be recommended to all who are interested in this phase of medical investigations.

Neuropathology†

THE development of neurology as an independent unit of the medical sciences, and the great advance which has been made in this highly specialized field has naturally been accompanied by a corresponding accumulation of facts concerning neuropathology, also a highly specialized subject.

Dr. Weil's book, therefore, should be welcomed, not only by the neurologist and neuropathologist, but also by the practitioner at large as a ready source of reference.

The purpose of the book is to present a review of the present status of neuropathology which is achieved in a very comprehensive and satisfactory manner.

The comprehensive character of the discussion is indicated by the Chapter headings which follow: Changes Through Autolysis and Fixation; Diseases of the Ganglion Cells; The Glia and Its Pathology; Pathology of the Myelin Sheaths and the Axis Cylinder; Anemic Softening; Arteriosclerosis; Inflammation; Infections; Intoxications; Injuries; Degenera tions; Tumors; and Congenital Malformations.

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*Recent Advances in Sex and Reproductive Physiology. By J. M. Robson, Research Fellow, Institute of Animal Genetics, University of Edinburgh. Cloth, pp. 249, 47 illustrations. P. Blakiston's Son and Co., Philadelphia, Pa.

†A Textbook of Neuropathology. By Arthur Weil, M.D., Associate Professor of Neuropathology, Northwestern University Medical School. Cloth, pp. 335, 260 illustrations. Lea and Febiger, Philadelphia.

An appendix contains a description of satisfactory methods for the autopsy and fixation of the central nervous system.

It is not to be expected that the perusal of this book will make of the reader a neuropathologist. The clinician may well refer to it for a better understanding of the relationship of the nervous system to disease in general; the pathologist will find it a useful and valuable reference; and to the neuropathologist it presents under one cover a succinct yet thorough survey of the information at present available.

The book evidences a wide experience and a thorough acquaintance with the subject and can be well recommended to those who are, or should be, interested in this subject.

The illustrations are not only numerous but excellently reproduced. An extensive bibliography and satisfactory index are appended.

The Medicolegal Necropsy*

THIS book should be of the greatest interest, not only to coroner's physicians, pathologists, and all who may be called upon to perform necropsies for medicolegal purposes, but also to the physician at large as a reliable and comprehensive survey of a very important subject.

The introductory chapter by Dr. F. E. Sondern reviews the faults of the present coroner's system and the following chapter by Dr. O. T. Schultz outlines the workings of the medicolegal system of the United States and the status of the medicolegal autopsy.

Dr. Charles Norris, Chief Medical Examiner of New York City, furnishes a chapter on The Medicolegal Necropsy and the manner in which it should be carried out is described by Dr. A. V. St. George. Toxicology in the Medicolegal Necropsy is described by A. O. Gettler, followed by a very comprehensive discussion of the findings in deaths from shooting, stabbing, cutting, and asphyxia by Dr. H. S. Martland, Chief Medical Examiner for Essex County. E. L. Miloslavich, of the Institute of Legal Medicine of Jugoslavia contributes an excellent discussion of the pathological anatomy of death by drowning.

An appendix presents the report of a committee on necropsies which should be read by every pathologist.

The high standing of all the authors assures the reader of an authoritative presentation based on extensive experience.

This small volume may well become a standard reference text and deserves, as it will doubtless receive, a wide circulation.

It contains so much and so varied information that it might well have been furnished with an index.

The book may be highly recommended without reserve.

Diabetic Manual for Patients†

DIABETIC manuals for the use of patients were started prior to the era of insulin, especially by Joslin. At that time the importance of carefully measured and controlled dietaries was great. Since insulin, the impression has become very general both among the laity and among physicians that as long as a patient takes his insulin he need not follow any carefully supervised dietary program. This makes for carelessness and in the end usually results in continued loss of tolerance. No one knows better than those whose special interest

*The Medicolegal Necropsy: A Symposium Held at the Twelfth Annual Convention of the American Society of Clinical Pathologists. Edited by Thomas B. Magath. Cloth, pp. 167. 63 illustrations. Williams and Wilkins Co., Baltimore, Md.

†Diabetic Manual for Patients. By Henry J. John, M.A., M.D., F.A.C.P., MAJ. M.R.C., Director of the Diabetic Department and Laboratories of the Cleveland Clinic. Second Edition. Cloth, pages 232, 1934. The C. V. Mosby Company, St. Louis, Mo.

is in the field of diabetes that for optimal results the patient still must know intimately the details of his disease and must carefully control his diet even though the diet is many times more generous than in the old starvation period.

The second edition of John's Manual would appear to fulfill excellently the need for which it was written. It tells the patient much about himself and goes into details such as the desirability or otherwise of marriage of diabetics, the relationship of obesity to the disease, the proper prevention of complications, acidosis, coma, gangrene and proper hygiene for the diabetic patient. The language is sufficiently simple so that any patient with a modicum of intelligence can read and understand. The illustrations are thoroughly descriptive, particularly those which accompany the food tables, and show, in red, the carbohydrate content of the various foods. This red danger signal stands out clearly with the result that the diabetic patient has no difficulty in a visual understanding of the need for avoiding this and that.

Dr. John has pioneered in the summer camps for diabetics. In discussing the value of this he emphasizes that the diabetic child is at a decided disadvantage in the summertime when other children may go to camp. And the mother is at a greater disadvantage because she must stay at home and continue to treat the child. The summer camp is a wonderful surcease for both mother and child. In the preinsulin days this was not a necessity among diabetic children because their lives were short at best. Now, when they are growing to adult life and usefulness, they must be prepared therefor.

The book should continue to fulfill in an excellent manner the purposes for which it was written.

The Compleat Pediatrician*

THIS is a book which can be recommended without reserve. In few specialties in the field of medicine will the physician be confronted with more diagnostic difficulties than in pediatrics for the very obvious reason that in this specialty he is to a very large extent dependent upon his powers of observation, his clinical skill in noting and eliciting the signs and symptoms of disease, and especially upon the degree to which he is prepared to utilize the processes of inferential deduction developed through reading, study, and past experience.

While, as the author comments in his preface, the best way to obtain a durable and useful knowledge of pediatrics—as well as, indeed, of any branch of medicine—is through the study of the patient, he recalls also Osler's saying that, "to study the phenomena of disease without books is to sail an uncharted sea, while to study books without patients is not to go to sea at all."

As the title page suggests, Dr. Davison set himself an ambitious and comprehensive task. Study of the book reveals that in its execution he has fallen but little, if anything, short of fulfilling his plan.

This book consists of Chapters: I, Symptoms and Signs of Disease in Children; II, Diseases, Differential Diagnosis, and Treatment; III, Preventive Measures and Child Care; IV, Administration of Fluids and Blood to Infants; V, General Suggestions for the Feeding of Normal Infants and Children; VI, Drugs and Prescriptions Frequently Used in Pediatrics; and VII, Laboratory Methods Frequently Used in Pediatrics.

An Appendix (B) presents an outline for pediatric histories and the physical examination of children, while Appendix C lists the contents of the pediatrician's bag.

While some one complained somewhere that of the making of books there is no end, Dr. Davison's book stands almost in a category by itself. Its plan is not only original but well carried out and the contents reflect not only an extensive and digested experience, but also a comprehensive acquaintance with and survey of the literature of pediatrics and may well be to the pediatrician what Roget's *Thesaurus* is to the writer.

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*The Compleat Pediatrician: Practical, Diagnostic, Therapeutic, and Preventive Pediatrics for the Use of Medical Students, Internes, General Practitioners and Pediatricians. By Wilburt C. Davison, M.D., Professor of Pediatrics, Duke University, School of Medicine. Cloth. pp. 260. Duke University Press.

The plan is that of a thesaurus permitting extensive and diversified cross reference. While the numbered paragraphs may present, to the novice, a peculiar and perhaps complicated appearance, the book is simple to use.

As always, the first requisite is a careful history and examination. Should the presenting symptom be, for example, headache, one finds this alphabetically listed in paragraph 16 of Chapter I. Under the causes, the reader is referred to the *common* causes as well as to *rare* causes, each bearing a paragraph number such as, for example, to typhoid-paratyphoid fever (851) meningococcus meningitis (531), sinusitis (775), eyestrain (359), etc. In these latter paragraphs will be found descriptions of additional signs and symptoms which should be present for diagnosis; references to other diseases which will cause similar symptoms; to various diagnostic laboratory measures; to the most useful therapeutic measures; and, finally, to any possible preventive measures.

The book, therefore, is exceedingly simple to use and remarkably and most commendably practical in the presentation of its contents. It abounds, moreover, in excellent concise tables especially in Chapter I.

The laboratory section is both comprehensive and practical as, indeed, is true of the book as a whole.

Dr. Davison is to be congratulated in having made a permanent addition to pediatric literature and one which deserves—as it will doubtless receive—an enthusiastic reception from the large and varied audience to which it is addressed.

To the laboratory worker, often called to interpret the laboratory findings in children, it should prove a most useful work of reference.

While it is questionable if there is anything in the field of pediatrics concerning which some mention will not be found in this book, emphasis is wisely laid upon the most important diseases encountered in children. Of these (as determined by incidence in 80,000 children representing 150,539 cases over a period of twenty years in the wards and dispensaries of Johns Hopkins Hospital and the Harriet Lane Home) there are 307, only 100 of which are important.

These 100 diseases cause 56 per cent of the deaths in children and can be prevented, and 21 per cent of the pediatric deaths, although responding to adequate treatment.

We repeat that this book may be recommended without reserve as an invaluable contribution to pediatric literature.

Physical Diagnosis*

CABOT'S *Physical Diagnosis* needs no introduction and the fact that it has now reached its eleventh edition is no surprise to those who are familiar with it—as who is not?

While it represents, as the author emphasizes and reiterates, the strictly personal viewpoint of the author, and while only that is included with which the author has had personal experience, this book is nevertheless a valuable, useful and practical contribution with which every practitioner and student of medicine should be familiar.

In thus presenting what he himself has found to be valuable in the study of disease, it is inevitable that the reader will encounter omissions, such as, for example, no mention of "poker spine" as an early sign of poliomyelitis under the discussion of "stiff back."

The book is a clearly written account of Dr. Cabot's own experience which is admittedly extensive, well told, and may well find a place in the library of every practicing physician.

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*Physical Diagnosis. By Richard Cabot, M.D., Professor of Clinical Medicine Emeritus, Harvard University. Edition 11, cloth, pp. 540, 317 figures. William Wood and Co., Baltimore, Md.

The Journal of Laboratory and Clinical Medicine

VOL. 20 ST. LOUIS, MO., NOVEMBER, 1934 NO. 2

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

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EDITORIALS

The Laboratory Diagnosis of Tuberculous Infection in Childhood

THE essential importance of early recognition of tuberculous infection in childhood has long been recognized but the diagnostic problem presented is not always easy of solution.

Symptoms may be indefinite, the roentgenographic picture and the results of tuberculin tests not always clear-cut, and the procedure commonly applied in the adult, the examination of the sputum for tubercle bacilli, is often inapplicable either because the children do not cough, or are unable to bring up sputum, or the sputum is swallowed.

Very naturally the search for these organisms then turned to the gastric contents or the intestinal discharges, the examination of specimens secured by gastric lavage being first suggested by Meunier[1] as long ago as 1898. Since then the procedure has been studied somewhat spasmodically by a number of investigators with somewhat variable results but, on the whole, showing that the method was sufficiently valuable to be borne in mind in the doubtful case.

While a positive response to the intracutaneous tuberculin test is of definite significance, the important fact, as regards the arrest, control, and dissemination of tuberculosis is to determine whether or not the case is open and active.

The tuberculous child with an active and open lesion discharging tubercle bacilli is obviously more of a menace to other children around it than one in whom these circumstances do not obtain; and it is to this phase of the question that attention has been directed within recent years, and to obtain evidence bearing upon it that the examination of gastric contents and feces for tubercle bacilli has again been resorted to by several groups of investigators.

Among the more recent reports is that of Gourley[2] who studied 59 cases by means of gastric lavage and animal inoculation. In 9 cases there were no demonstrable roentgenographic lesions, 50 presented such evidence, and all gave a positive tuberculin test.

Tubercle bacilli were present in the gastric contents of 28 or 47.4 per cent of the whole series and in 28 or 56 per cent of the 50 having demonstrable lesions.

That calcification of the parenchyma of the lung or regional lymph nodes is not indisputable evidence of entire arrest and inactivity was suggested by the fact of 12 children showing evidence of calcification in the film; 5 had tubercle bacilli in the gastric contents. Gourley concludes from her study that all types of parenchymal lesions in childhood tuberculosis, including McPhedron's "C" group, are open cases.

A series of 60 cases examined by Mishulow, Kereszturi, and Hauptman[3] gave 10 per cent positive findings in children apparently well when examined, 54.5 per cent positive findings in those slightly ill, and 63.1 per cent positive findings in children who were very ill. Of those with nondestructive parenchymatous lesions 24 per cent were positive while 83.3 per cent were positive in the group showing destructive parenchymatous pulmonary lesions.

Negative findings were encountered in all cases with only positive intracutaneous tests, hilum lymph node tuberculosis, pleurisy with effusion and tuberculous peritonitis.

In this series tubercle bacilli were found in the feces in 12 cases, but in variable numbers, in one case so few that they were only detected when the smears were reexamined after guinea pigs proved to be positive.

Although examination of the feces can be done repeatedly without discomfort to the patient the results are not quite as good as those from examination of the gastric lavage, and the procedure is, moreover, somewhat more complicated.

In the interpretation of such findings possible sources of error must, of course, be remembered and taken into account if they cannot be eliminated.

Thus, tubercle bacilli may be ingested with raw milk, tuberculosis of the stomach may exist but is so rare as to be of negligible importance, tuberculosis of the tonsil is also a somewhat rare possibility, and there may be tuberculous foci in the sinus or middle ear.

In considering the significance to be attached to the demonstration of tubercle bacilli in the gastrointestinal tract in children Nalbant[4] points out that there are various possible sources, among which are:

A tuberculous infiltration in the lung, such lesions usually being demonstrable clinically or by x-ray.

A receding type of childhood tuberculosis in which the lesions may have cleared roentgenographically though not pathologically.

A caseating tracheobronchial lymph node which may have broken into the trachea or a bronchiole or even through the esophagus.

Other possibilities are, as has already been noted, the presence of an upper respiratory tract tuberculous infection which, however, is uncommon; tuberculous enteritis or gastritis, quite uncommon, and the presence of a tuberculous growth in a bronchus or bronchiole. It has also been suggested by some, Mikulowski,[5] for example, that the source of tubercle bacilli in the feces may be infected bile which has regurgitated into the stomach. This, of course, could only be accounted for either by assuming a primary gallbladder lesion, certainly an unusual occurrence, or by the assumption that the infection of bile was the aftermath of a tuberculous bacteriemia. In the latter case the complete escape from tuberculous meningitis or a generalized tuberculosis would be difficult to explain.

On the other hand, it is fair to assume that many, if not the great majority, of cases of tuberculous pleurisy, tuberculous bone and joint disease, or Pott's disease may well be localizations of a former bacillemia.

The problem is one well worthy of further study, not only from the standpoint of furnishing an additional avenue for the laboratory diagnosis of tuberculosis in childhood, but also, because of the clinical and social significance of the results obtained, which may only be evaluated after extensive study.

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—*R. A. K.*

Staff Meetings and Statistics

ONE of the requirements for an "approved" hospital is that there shall be a monthly Staff meeting at which the work done and the results secured by various departments shall be discussed and evaluated.

The principle underlying this provision is, without doubt, wise and well founded but if the reports thus made obligatory are to be productive of the results desired they must be something more than mere statistical recitals, and the discussion which they are intended to provoke must comprise something more than an exchange of compliments.

As every one knows, the value of statistics depends, not merely upon their volume, but upon the data from which they are compiled, the honesty with

which they are gathered, and the sincerity and care with which they are interpreted.

For the intelligent interpretation of statistical data one must take into consideration, not only the figures themselves, but also the many and varied aspects of the cases to which they refer. For it is obvious that during certain periods, for example, the admissions to a hospital may include many patients whose condition makes recovery problematical, many patients who, despite the fact that they may be justly regarded as unavoidably bad risks, must nevertheless be subjected to operation with an effect which may be foretold with some confidence upon the mortality statistics of the surgical service in question.

Nevertheless, the not uncommon practice of briefly and sometimes even cursorily reciting the number of cases of this, that, and the other thing together with a bald statement that so many were discharged "cured" or "improved," while it may conform to the letter of the law, falls far short of real compliance with the end in view.

The real purpose, it may be suggested with some justice, is to determine, not merely what happened, but why did it happen and, what is still more important, could it have been avoided?

Although speaking of operative mortality only, Lahey* has enunciated principles which are equally applicable to medical services as well and might well be widely disseminated.

"While there will be certain years," he says, "in which many more serious cases will require operation than in others, and there will be others in which unavoidably bad risks must be accepted, nevertheless, the yardstick whereby one may measure surgical skill, surgical judgment, and the protection with which the patient is surrounded, is that of mortality."

This, of course, partakes somewhat of the nature of a truism but Lahey adds these significant qualifications:

"Mortalities are in some measure related to the surgeon's state of mind. If a surgeon assumes the position that a certain number of patients must die anyway following surgical procedures since, to be given a real chance of cure, particularly in carcinoma, radical procedures must be done, then it naturally follows that a certain number of mortalities will occur. . . . If one is prepared to expect mortalities, he has in some measure compromised his conscience to their occurrence. In my own opinion, the attitude should be that patients should not die following surgical procedures, that when they do die following surgical procedures, one's attitude, with reasonable exception, should be that an error in judgment has been made, either in advising operation at all, in the extent of the operation, in some of the technical procedures, or in the way of pre- or post-operative management. . . .

"It is extremely easy in surgery, particularly when dealing with patients who are in a desperate condition, to justify in one's mind desperate risks, but very rarely, in my experience, do desperate risks accomplish very much which is worth while. . . . When one knows and frankly faces the mortality rate this has the advantage of making one acutely aware of his errors in judgment and

*Lahey, F. H.: Operative and Mortality Statistics of The Lahey Clinic for 1932, Bull. Lahey Clinic, 1932.

stimulating every one who is connected with operative procedure to his utmost efforts, pre- and postoperatively, to prevent the occurrence of a mortality."

These quotations present and embody an attitude equally applicable in principle to medical mortality statistics as well as surgical and deserve general consideration.

The development of such an attitude would without doubt remove from many Staff meetings the cut and dried and stultifying acceptance of mortality statistics with resigned equanimity and lead, on the contrary, to the awakening, not of acrimonious criticism, but of a spirit of earnest inquiry. To repeat what has already been said above, it is not the recital of what happened that is important and profitable but why did it happen and, more particularly, could it have been avoided?

It is hardly conceivable that there exists a physician or a hospital staff with the temerity to assert or the credulity to believe that, individually or collectively, they have never been guilty of an error of judgment, a failure to reach the proper diagnosis, an omission of something which might have been useful in the study of a particular case. How many can honestly deny an excessive and ill-warranted use, for example, of laboratory methods neither suggested nor indicated by the clinical data, the place of which they are all too often made to take?

How many times must honesty compel us to sigh with the unknown who regretted that his "foresight was not as good as his hindsight"?

And how many times are Staff meetings characterized by any such frank admissions or discussions?

It is particularly in the hospital of small or moderate size that such discussions would be most productive of benefit to all concerned, as it is also particularly in such hospitals that they are most often neglected.

The full function and entire duty of a hospital cannot be achieved by routine work, nor can it be expressed solely by the accumulation and recital of statistics relating to admissions, operations, and discharges.

Unless the data so accumulated are so studied and analyzed—critically, if need be, that continual improvement in methods and ability results, if not achieved is at least sought for, then Staff meetings, though complying with the letter of the law, are more apt to be boring and stultifying than inspiring or profitable.

Mistakes are, in the ordinary course of human events, more or less inevitable. It is not the mistake but the failure to learn from it which is disgraceful and leads to its repetition.

It is from our failures that we should learn rather than from our successes. And it is upon the discussion and analysis of our failures that we should spend our time leaving our successes to speak for themselves.

—*R. A. K.*